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VOLUME 7 NUMBER 3

JUNE 1917

Phytopathology

Official Organ of the

American Phytopathological Society

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PUBLISHED BIMONTHLY FOR THE SOCIETY

OFFICE OF PUBLICATION

2419-21 Greenmount Ave., Baltimore, Md.

Entered as second-class matter May 16, 1913, at the post office at Baltimore, Maryland, under the Act of March 3, 1879

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The Official Organ of the American Phytopathological Society

The Journal Treats of Diseases to Which Plants Are Subject and the Remedies for Such Diseases

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PHYTOPATHOLOGY

VOLUME VII NUMBER 3

JUNE, 1917

STUDIES ON BACTERIUM SOLANACEARUM[1]

E. E. Stanford and F. A. Wolf

With One Figure in the Text

Studies on the wilt diseases caused by *Bact. solanacearum* have been in progress at the North Carolina Agricultural Experiment Station since 1903. A recent bulletin (4) dealing primarily with remedial and palliative measures for tobacco wilt contains the results of certain of these investigations. In the present paper are presented data bearing (1) on the distribution within North Carolina of the disease on tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*), (2) on cultural studies to determine the identity of the strains from the several hosts and (3) on the results of cross inoculations.

DISTRIBUTION OF BACT. SOLANACEARUM WITHIN NORTH CAROLINA

Tobacco wilt has probably been present within the state for at east twenty-five years, but occurs in only a small proportion of the lands devoted to tobacco culture. The disease was first definitely recognized in the southern portion of Granville county in 1902. Records indicate that the disease has yearly continued to spread so that it now occurs in eleven counties, namely: Granville, Durham, Vance, Wake, Franklin, Ashe, Guilford, Greene, Davidson, Chatham and Yadkin.

The losses in the first four of these counties are confined to the southern portions of Granville and Vance and the northern portions of the adjacent counties of Durham and Wake. In this area the disease is so generally destructive that the growing of tobacco has been abandoned on many farms. The disease is confined, however, to relatively small areas in the seven remaining counties.

[1] Acknowledgment is hereby made to the several members of the Department of Botany and Plant Pathology of North Carolina Agricultural College and Experiment Station, who, since the inception of these investigations, have aided in the work.

It is a striking fact that collections of bacterial wilt of tomato which have been very generally made throughout the State show that the disease on tomatoes occurs in thirty-nine counties of the state. It is realized, of course, that the disease on both tobacco and tomatoes may not have been reported from all localities in which it may occur, yet the fact remains that the disease on the two hosts is not coextensive. No adequate explana-

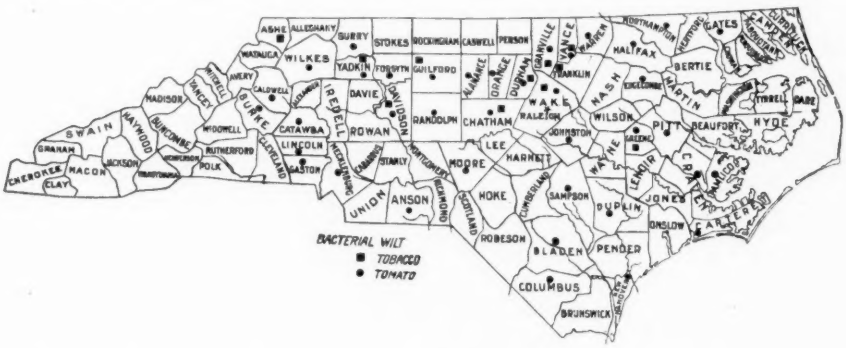

FIG. 1. MAP OF NORTH CAROLINA SHOWING THE KNOWN DISTRIBUTION OF BACTERIUM SOLANACEARUM ON TOBACCO AND ON TOMATO

tion is at hand to account for this, in view of the fact that reciprocal inoculations are so easily effected and that the strains of *Bact. solanacearum* from tobacco and tomatoes are identical as shown by the cultural studies of several investigators and confirmed by studies to be presented later in this paper.

It might be added, that no data have accumulated relative to the distribution of this parasite on other solanaceous hosts and that the disease on peanut (*Arachis hypogœa*) has been observed in one locality only.

CULTURAL STUDIES

In view of the fact that in certain sections, *Bact. solanacearum* is not present on all of the hosts which are known to be subject to attack, a study by means of artificial cultures was made to determine the identity of the strains from the more common hosts. Several investigators, among whom may be mentioned Smith (5 and 6) and Honing (6)[2] have previously reported extensive studies on Bact. solanacearum in artificial culture and regard the organism from the several hosts as identical. Nevertheless,

[2] References to the publications of Honing, Hunger, and Uyeda are given in Volume 3 of Smith's Bacteria in Relation to Plant Diseases. The authors have not seen these publications, but only the abstracts given by Dr. Smith. Bibliographies of *Bact. solanacearum* on p. 218–219 and 270–271.

parallel cultures of the organism isolated from tobacco, potato, (*Solanum tuberosum*) tomato, eggplant (*Solanum melongena*), peanut, ragweed (*Ambrosia artemisiifolia*) and *Eclipta alba* have been made. These isolations were made from various parts of the hosts and from plants from several localities within the state. In general, the cultural variations which appeared between the strains from the different hosts were no greater than those from strains all of which came from the same host. Since, however, certain additiona[1] facts are brought to light, a brief account of the growth in culture is pertinent and is herewith given.

Colonies on agar plates usually become visible within thirty-six to forty-eight hours at 28°C. At a temperature 4 to 5° lower, they may be scarcely noticeable at the end of seventy-two hours. They are at first white, wet-shining and opalescent, circular in outline, slightly raised and with smooth margin. Colonies become 3 to 5 mm. in diameter in five to six days. They soon become distinctly opalescent-blue by transmitted light, when the plates are held some distance from a poorly lighted reflecting surface. When the colonies are viewed with the blue sky for a background, they are distinctly brown with faint concentric rings. Later, they become very markedly brown by reflected light. The pigmentation originates at the center of the colony, spreads toward the margin and is diffused somewhat into the agar. The color is more intense at the center of the colony. Subsurface colonies are globose or lenticular, much smaller than the surface colonies and become brown much sooner than do the surface colonies.

The growth on agar slants develops slowly, is filiform at first and usually spreading at the bottom of the slant. It is slightly elevated and the margin is entire or wavy. Colors and pigmentation develop similar to those in plate colonies with considerable variation in time of appearance and intensity of pigmentation which may vary from scarcely perceptible to brownish black. When Witte's peptone is used, a deeper pigmentation develops than when Difco peptone is employed. When the colonies become blackened, the organism is no longer viable. It appears to retain its vitality for about four and one-half months on agar but rapidly loses its virulence on this medium.

The appearance of colonies on litmus lactose agar slants is similar to those on nutrient agar. The litmus is at length slightly reduced. Pigmentation is first noticeable in three to four weeks and a brown stain soon becomes diffused throughout the agar and masks its color. Portions of the substratum which are not brown become sky-blue by diffuse light and reddish plum-colored by transmitted light.

The appearance of this organism in bouillon cultures is somewhat variable. A rather uniform clouding develops within twenty-four hours

which becomes so intensely opaque within a week as to make it impossible to see through a bouillon tube when the tube is placed immediately in front of an object. Numerous pseudogloeae may appear forming flocculent particles on the surface. No pellicle nor ring is formed, but a thin, opalescent scum appears on the surface. A dirty white, viscous precipitate develops within a week or two. Within four to six weeks, this precipitate will have become dense and the supernatant bouillon will have become clear at which time the organism is no longer viable. Various degrees of pigmentation may occur, being more intense with Witte's than with Difco peptone. Tubes of the bouillon made with the former become brownish black on long standing.

On potato plugs, the growth is spreading, thin or slightly raised, white or flesh-colored at first but rapidly becomes brown, often pitch-black. The surrounding liquid becomes clouded and brown. The organism is short-lived on this medium and commonly loses its vitality within a week. The virulence of *Bact. solanacearum* on artificial media is best retained on potato plugs, but transfers must be made at intervals of about two days.

When grown on milk, there is no peptonization nor precipitation of casein. A slight viscidity and the odor of putrefaction are developed and the medium at length becomes brown and alkaline.

The organism causes a partial clarification of litmus milk with a deepening of the blue color which appears reddish by transmitted light. A slight dirty white precipitate is formed, which becomes brown on long standing. The bacteria may remain viable for five months on this medium.

The surface colonies on gelatin are small, circular, white and wet-shining. Submerged colonies are globose and yellowish to brownish. Growth along the line of the stab on gelatin is white, later becoming brown, filamentous and best at the surface of the medium. No liquefaction occurs.

There is no evident growth on Cohn's solution in four weeks. On Uschinsky's solution, growth ranges from none to feeble with slight clouding.

In dextrose broth, a copious growth develops in the open arm, extending only to the base of the closed arm. An abundant, rather viscous precipitate appears. The medium at length becomes brown in the open arm and is strongly alkaline with no evolution of gas.

Cultural characters on saccharose broth are similar to those on dextrose with a less marked tendency to the development of a brown color.

Growth is feeble on lactose broth with little sedimentation and little or no brown color is developed, even after seven weeks. The acidity of acid broth is diminished but neutral broths are not rendered alkalin. On mannit, the growth characters are similar to those on lactose. A very

copious growth ensues in glycerin broth with a marked development of brown color. Growth on maltose is similar to that on dextrose. Nitrates and ammonia are formed in moderate amount in nitrate solutions.

Growth is much delayed and diminished in hydrochloric acid +25 Fuller's scale, and is entirely inhibited at +30. No growth occurs in double strength bouillon rendered +33 acid by the addition of expressed tomato fruit juice. Smith (6) reports growth in +33 acid of beef juice. The optimum reaction lies between +10 and +15.

Growth is slight or none in bouillon −5 with sodium hydroxid. No growth occurred in −10 sodium hydroxid. The organism is little retentive of vitality on culture media. Milk appears to be the best medium for long continued growth on *Bact. solanacearum*. The organism may remain viable for two months in sterilized distilled water. No evidence of diastatic activity was found when the organism was grown on potato plugs.

A considerable number of special media have been prepared, among which are soil extract, casein agar, Heyden's Nährstoff agar, potato agar, potato leaf agar, and tomato leaf agar. No growth of diagnostic significance developed on any of these media.

Bacterium solanacearum is very short-lived in mixed cultures. Honing (6) noted a marked antibiosis between the wilt organism and *B. mesentericus* as well as other species plated from wilted tobacco. In our studies also, various bacteria have been found to replace *Bact. solanacearum* in decaying, wilted plants. Five strains of yellow chromogens isolated from diseased tomatoes, tobacco and peanuts were found in the fall of 1915 to exhibit marked antagonism to the wilt organism. In intersecting streaks on agar plates, the chromogens tended to crowd out the parasite. The presence of *B. mesentericus* and other soil inhabitants appear never to be so antagonistic, however, as to eliminate *Bact. solanacearum* from infested soils.

Thus far attempts to isolate the parasite directly from infested soils have been unsuccessful. This is due in part at least to the fact that *Bact. solanacearum* is inhibited by other soil inhabitants which develop on the plates. Honing (6) however, succeeded in isolating it on plates from dilution cultures of well water.

CROSS-INOCULATION EXPERIMENTS

Bacterium solanacearum has previously been shown to attack members of eight widely separated families, Urticaceæ, Leguminosæ, Tropaeolaceæ, Euphorbiaceæ, Verbenaceæ, Solanaceæ, Pedeliaceæ and Compositæ.

The organism was first described by Erwin F. Smith (5) in 1896 as

the cause of a wilt disease of tomato, eggplant and potato and he successfully inoculated *Solanum nigrum*, *Datura stramonium*, *D. metelloides*, *D. fastuosa*, *D. cornucopia*, *Physalis crassifolia*, *P. philadelphica* and *Petunia* (hybrid).

Several investigators, among whom are Hunger (6), Stevens and Sackett (7) and Uyeda (6) have reported a wilt disease of tobacco. Honing (6) in 1910 first reported this organism as the cause of disease in plants outside of the Solanaceæ. He found it in *Pouzolzia* sp., *Physalis angulata*, *Indigofera arreeta*, *Arachis hypogœa*, *Mucuna* sp., *Acalypha boehmeroides*, *Ageratum conyzoides*, *Spilanthes acmella*, *Pluchea indica*, *Blumea balsamifera*, *Synedrella nodiflora* and *Tectona grandis*. He also successfully inoculated several ornamental varieties of *Nicotiana*, *Capsicum annuum* and *Sesamum orientale*.

A wilt disease upon peanut was subsequently reported from North Carolina (2) and later studies (3) in this state added two composites, *Ambrosia artemisiifolia* and *Eclipta alba*, to the list of naturally infected hosts.

A wilt of nasturtium (*Tropœolum majus*) caused by *Bact. solanacearum* was reported from Maryland by Bryan (1). She succeeded in inoculating also the common cultivated Ageratum and Verbena.

The artificial inoculation experiments conducted at the North Carolina Experiment Station prior to 1913 were confined primarily to solanaceous plants. When in the summer of 1912, it was found that peanuts are subject to attack by *Bact. solanacearum*, this host was successfully inoculated with strains from tobacco, peppers and peanuts. The strains from peanuts were also found to be pathogenic to tobacco.

During the season of 1914, *Bact. solanacearum* was isolated from diseased ragweeds (*Ambrosia artemisiifolia*) and subsequently found to be productive of wilt on tobacco, tomato, potato, *Eclipta alba* and garden nasturtium (Tropæolum). The reciprocal inoculations upon ragweed with strains from tobacco, tomato, potato and *Eclipta alba* were rather unsuccessful. No systemic invasion resulting in death, but merely a local occlusion and blackening of xylem elements occurred in inoculated plants.

In the fall of 1915, strains isolated from wilted *Eclipta alba* were successfully inoculated into tomatoes, potatoes, tobacco, garden nasturtiums and *Eclipta alba*. A rather more comprehensive series of inoculations on cultivated and wild species was instituted in 1916, the results of which are herein briefly summarized.

Method of inoculation. The strain of *Bact. solanacearum* employed in making the initial inoculations was obtained by the poured plate method, from wilted tobacco plants from Creedmoor, North Carolina. As soon

as the organism had developed on these poured plates, transfers were made to potato plugs. Repeated transfers at intervals of one to three days were made on this medium. The pigmentation on old cultures on agar and on potato plugs was regarded as sufficiently characteristic to establish the identity of the wilt organism. Inoculum from one- to three-days-old cultures on potato plugs was used in all of the inoculations. Inoculations were made by pricking the plants near the tips of the branches and inserting the inoculum. A number of check plants, either uninjured or pricked with a sterilized needle were used in the case of each species tested. Since it was known that *Bact. solanacearum* loses its virulence even though repeated transfers are made, no attempt was made to use the original strain from tobacco in all of the inoculations. Instead, isolations from certain of the inoculated species were used in continuing the series of inoculations. Some differences in virulence appeared in strains which had passed through different hosts but no such marked decrease occurred as when the organism is repeatedly transferred on culture media.

In general, young, vigorously growing plants were used in these tests, although in some cases, rather mature plants were employed. The cultivated species were grown either in the greenhouse or in small experimental plats at West Raleigh, North Carolina and the weeds grew in waste places where they could be kept under observation for the necessary length of time. In general, as soon as inoculated plants showed symptoms of disease, they were examined microscopically to determine the presence of bacteria within the tissues at points remote from the point of inoculation. The organism was then reisolated by the poured plate method, and its identity established by the characteristic growth on agar and potato plugs. As supplementary evidence, the reisolated organism was inoculated into tomatoes or tobacco.

The accompanying diagram of the plan of these cross-inoculation experiments has been so arranged as to show at once the source of the inoculum, the result of the inoculation and the number of plants inoculated.

Results. When comparison is made with the host species previously enumerated, it will be seen from this tabulation of the results of cross inoculations that the following plants have heretofore been unreported as subject to attack by *Bact. solanacearum: Stizolobium niveum, Tropæolum lobbianum, T. peregrinum, Croton glandulosus* var. *septentrionalis, Impatiens balsamina, Verbena erinoides, Lycopersicon cerasiforme, L. pyriforme, Browallia demissa, Physalis alkekengi, Schizanthus pinnatus, Salpiglossis sinuata* and *Martynia proboscidea.* Twelve of these species belong to families representatives of which had hitherto been known to be subject to attack and one species, *Impatiens balsamina,* belongs to an additional family.

TABLE 1

Diagram of cross inoculations with Bacterium solanacearum[1]

- Nicotiana tabacum
  - xAMBROSIA ARTEMISIIFOLIA, 30
    - ooAmaranthus gangeticus, 10
    - ooAmaranthus caudatus, 10
    - xIMPATIENS BALSAMINA, 20
    - xxSolanum carolinense, 2
    - *xTropaeolum lobbianum*, 4
    - xTropaeolum majus, 4
    - ooHelenium tenuifolium, 11
    - xCROTON GLANDULOSUS, var. SEPTENTRIONALIS, 28*
    - ooBoehmeria cylindrica, 10
    - ooImpatiens fulva, 10
    - ooStizolobium niveum, 2
    - ooPhysalis alkekengi, 1
    - xxERIGERON CANADENSIS, 18
    - aAcalypha virginica, 20
    - oEuphorbia nutans, 10
    - xxAmbrosia artemisiifolia, 10
    - xx*Solanum carolinense*, 10
    - xBROWALLIA DEMISSA, 22
    - xxStizolobium niveum, 4
    - oPhysalis angulata, 4
    - xMARTYNIA PROBOSCIDEA, 2
      - xLycopersicon cerasiforme, 8
      - x*Salpiglossis sinuata*, 2
    - oVerbena venosa, 4
    - ooPetunia (hybrid), 6
    - ooDatura cornucopia, 2
    - ooDatura fastuosa, 6
    - ooChenopodium botrys, 3
    - xLYCOPERSICON CERASIFORME, 4
      - xxStizolobium niveum, 8
    - ooNierembergia gracilis, 6*
    - ooPhytolacca decandra, 4
    - ooSida spinosa, 5
    - xxBIDENS BIPINNATA, 10*
      - xTropaeolum peregrinum, 2
      - oSchizanthus pinnatus, 2
    - xSolanum nigrum, 1
    - xSolanum melongena, 2
    - ooPhysalis alkekengi, 4
    - xEclipta alba, 10*
    - xxStizolobium niveum, 2
    - ooAdicea pumila, 10
    - ooBoehmeria cylindrica, 10
      - x*Impatiens sultana*, 1
      - xMartynia proboscidea, 2
    - xPHYSALIS ALKEKENGI, 6
  - xDATURA TATULA, 10
    - xSTIZOLOBIUM NIVEUM, 4
      - xVERBENA ERINOIDES, 4
      - xTropaeolum peregrinum, 2
      - x*Schizanthus pinnatus*, 2

[1] All species within a brace were inoculated from cultures from the species appearing in small capital letters within the preceding brace. The wilt organism has been isolated from all species appearing in italics and successfully reinoculated into either or both tobacco and tomatoes. The character before the various species indicates the nature of the injury. *x*, signifies wilt or serious injury; *xx*, bacteria present in considerable numbers, but productive of no external evidence of injury; *a*, no injury, bacteria present only near the point of inoculation, not reisolated but the expressed ooze when used as inoculum, resulted in infections on tobacco; *o*, bacteria remain viable for a considerable time near the point of inoculation with only local injury of tissues, no reisolation; *oo*, no demonstrable infection of any sort. The numerals following the name of the hosts denote the number of plants inoculated. The asterisk (*) following the numeral indicates that strains from more than one host have been used in these inoculations with the development of similar symptoms.

It may also be noted from the tabulation that no infection resulted in *Stizolobium niveum* and *Physalis alkekengi* when inoculated with the organism isolated from wilted *Impatiens balsamina*. Further, no demonstrable infection resulted in the case of *Petunia* (hybrid), *Datura cornucopia, D. fastuosa,* and *Physalis alkekengi* when the isolations were made from wilted *Browallia demissa*. Smith (6) had previously shown the first three of these forms to be subject to attack. Since *Stizolobium niveum* wilted when *Datura tatula* was the source of the inoculum and *Physalis alkekengi,* when wilted *Verbena erinoides* was employed, it is indicated that virulence is influenced by the host plant.

In the following species, little or no external injury resulted from inoculation, but the vascular tissues were found to be invaded: *Euphorbia nutans, Solanum carolinense, Physalis angulata, Impatiens sultani, Bidens bipinnata* and *Erigeron canadensis.*

Inoculated plants of *Ambrosia artemisiifolia* and *Eclipta alba* wilted thus confirming previous studies (3).

In general, it can be said that the external symptoms and pathological histology of the plants which were artificially inoculated in these studies differed in no essential particular from those of other species which have previously been reported as hosts for *Bact. solanacearum*. The species of Tropæolum, Lycopersicon, Browallia and Eclipta tested are to be regarded as very susceptible, whereas, *Stizolobium niveum* and *Physalis alkekengi* appear to be very resistant. That *Stizolobium niveum* is highly resistant is shown by the fact that in a field test at Creedmoor, North Carolina, no demonstrable infection developed in any of the plants grown in wilt-infested soil. It is interesting to note that when young ragweed plants grown in the greenhouse were inoculated, they quickly succumbed to wilt, while numerous individuals grown out of doors when inoculated with the same strain showed no external symptoms of disease. Little external evidence of disease developed in rather mature plants of Croton but young plants were easily wilted. In the case of *Impatiens balsamina,* the foliage became slightly wilted, some distortion of the stems occurred and adventitious roots were formed. The discoloration of the vascular bundles of the stems showed through the cortical tissues as brown streaks. This species was found to wilt slowly when inoculation was effected by potting plants in infested soil. The discoloration of the vascular system is externally visible in wilted stems of *Eclipta alba* and the leaves become characteristically crisp and blackened.

The economic bearing of these additional weed and cultivated host plants for *Bact. solanacearum* upon the problem of wilt control is at once apparent when it is indicated that certain of these forms, namely; *Erigeron canadensis, Ambrosia artemisiifolia, Euphorbia nutans, Croton glandulosus,*

and *Solanum carolinense* are widespread in cultivated fields in the State. *Eclipta alba* is often found in ill-drained lands. *Datura tatula* and *Bidens bipinnata* are not uncommon weeds about farm buildings and lots. The Lycopersicons and *Martynia proboscidea* are locally rather common in gardens. The Tropæolums, Verbena, Impatiens, Browallia, Schizanthus, Salpiglossis and *Physalis alkekengi* are more or less commonly grown as ornamental plants. The results with velvet beans (*Stizolobium niveum*) which is related to Mucuna mentioned by Honing (6) are significant since this crop is becoming of considerable importance in the South. At least, it cannot be recommended that velvet beans be grown in a rotation system in soils infested with *Bact. solanacearum*.

SUMMARY

1. A wilt of tobacco caused by *Bact. solanacearum* has been observed in North Carolina in eleven counties and a tomato wilt caused by the same organism has been noted in thirty-nine counties.

2. Previous cultural studies on the identity of *Bact. solanacearum* from various hosts are confirmed since the variations which appeared in the strains from tobacco, potato, tomato, eggplant, peanut, ragweed and *Eclipta alba* were no greater than in strains all of which came from the same host.

3. A new family of phanerogams, Balsaminaceæ, has been added to the number previously reported to contain host species of *Bact. solanacearum*. Members of nine families are now known to be subject to attack by this organism. Thirteen additional species of plants, classified as follows showed well-defined wilting or serious injury:

Leguminosæ	*Stizolobium niveum*
Tropæolaceæ	*Tropæolum lobbianum, T. peregrinum*
Euphorbiaceæ	*Croton glandulosus* var. *septentrionalis*
Balsaminaceæ	*Impatiens balsamina*
Verbenaceæ	*Verbena erinoides*
Solanaceæ	*Lycopersicon cerasiforme, L. pyriforme, Browallia demissa, Physalis alkekengi, Schizanthus pinnatus, Salpiglossis sinuata*
Pedeliaceæ	*Martynia proboscidea.*

No outward signs of disease developed in the case of six other species in which the organism multiplied rapidly within the vascular portions. Five of these species are previously unreported, namely:

Euphorbiaceæ	*Euphorbia nutans*
Solanaceæ	*Solanum carolinense*
Balsaminaceæ	*Impatiens sultani*
Compositæ	*Bidens bipinnata, Erigeron canadensis.*

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SPARASSIS RADICATA, AN UNDESCRIBED FUNGUS ON THE ROOTS OF CONIFERS

JAMES R. WEIR

WITH FIVE FIGURES IN THE TEXT

In August, 1912, the writer collected several specimens of a species of Sparassis growing on the roots of various conifers in the Priest River Valley, Idaho. Lloyd, to whom specimens were sent, pronounced it an undescribed species. Cotton of the Pathological Laboratory at Kew who was advised by Lloyd of the writer's specimens stated that the plant was unknown to him. Since collecting the first specimens, the writer has studied the plant in several regions of the Northwest and finds that of the many peculiarities of the species the most surprising discovery is its evident parasitism on the roots of conifers. Although this fact was noted in 1912, it was not until the plant was carefully studied in its relation to its several hosts that this phase in its life history could be satisfactorily determined.

DESCRIPTION OF THE FUNGUS

Since the fungus does not agree with any known member of the genus, it is described as new.

Sparassis radicata n. sp.

Fruiting structure large, 12 to 22 cm. broad, 10 to 16 cm. high, dilated above, compact, fleshy, tough, whitish, creamy yellow with age, branched; branches numerous, horizontal or vertical, anastomosing, sometimes forming labyrinth-like cavities, more often compactly arranged, very thin, fan-shaped with wavy, sometimes deeply lobed margins, occasionally striated, amphigenous or unilateral, depending on the position of the branch; stalk, sclerotioid, tuberculate, firm, solid, sometimes branched, 20–30 cm. long, 5–8 cm. broad; spores, (50) range 2.8–4.0 × 2.8–5.5 μ, standard size 3.9 × 5.1 μ, ovoid, hyaline.

Type locality. Priest River, Idaho.

Habitat. Living roots in coniferous trees.

Range. Oregon, Idaho, Washington, Montana, and British Columbia.

Type material. In the Office of Investigations in Forest Pathology, Bureau of Plant Industry, Missoula, Mont.

GENERAL MORPHOLOGY AND TAXONOMY OF THE GENUS SPARASSIS

The genus Sparassis was established by Fries[1] and placed in the Clavariaceæ because of its frondose habit, fleshy consistency, and the belief that the spores were produced on all surfaces of the sporophores. It has recently been shown by Cotton[2] that the hymenium of Sparassis is not amphigenous but that the flattened branches with the exception of those in the center of the sporophore, are unilateral. On the basis of the flattened sporophore and the inferior hymenium Cotton suggests that Sparassis should be removed from the Clavariaceæ and placed in the Thelephoraceæ. He points out that in the Merisma section of the genus Thelephora are species with upright, partly unilateral sporophores either terrestrial or growing on wood which in many respects have the characters of Sparassis. In points of smoothness of the hymenium he further suggests that Sparassis is allied to Stereum but since the relationship to Stereum is not very close sees no reason why the genus Sparassis should not be transferred to the Thelephoraceæ without reference to any particular genus. Sparassis would then be distinguished as a genus of the Thelephoraceæ having fleshy, flattened, horizontal or vertical anastomosing branches with unilateral structures. The same view is entertained by Lloyd[3] who thinks the definition as laid down by Fries "fertile on both sides" should be corrected. Whether or not this view should be adopted in view of the fact that there is considerable irregularity in the formation of a unilateral sporophore is doubtful. In young sporophores of *Sparassis crispa* (Wulf.) Fr. examined by the writer, also of *Sparassis radicata*, the hymenium is by no means confined to the lowermost portion of the flattened branches but is found more or less uniformly over all free surfaces. This is particularly true, as Cotton points out, for those branches in the center of the sporophore but with a more pronounced unilateral structure toward the periphery. The hymenium of *Sparassis radicata* is formed very rapidly on the reverse side of the peripheral lobes when changed from their origninal position. A few specimens with unusually vertical lobes showed an amphigenous hymenium throughout making it seem probable that the lobes only become unilateral when they develop in a position allowing the influence of gravity to be more active on one side than another. There are, however, very few unilateral fungi, if any, that, under proper conditions of growth, will not when reversed develop the hymenium on the upper side.

[1] Fries. Systema mycol., I, p. 462.

[2] Cotton, A. D. On the structure and systematic position of Sparassis. Trans. British Myc. Soc. **1911:** 336–339.

[3] Lloyd, C. G. Letter No. 61, note 400.

Both Quélet[4] and Patouillard[5] noted the affinities of Sparassis with certain groups in the Thelephoraceæ but apparently without definite knowledge of the hymenial development in the genus. Somewhat later Maire[6] separated Sparassis from the Clavariaceæ making it the type of a special family, the Sparassideæ. Although Maire's classification was adopted by Lotsy,[7] critical work on the development of the hymenium, permanency

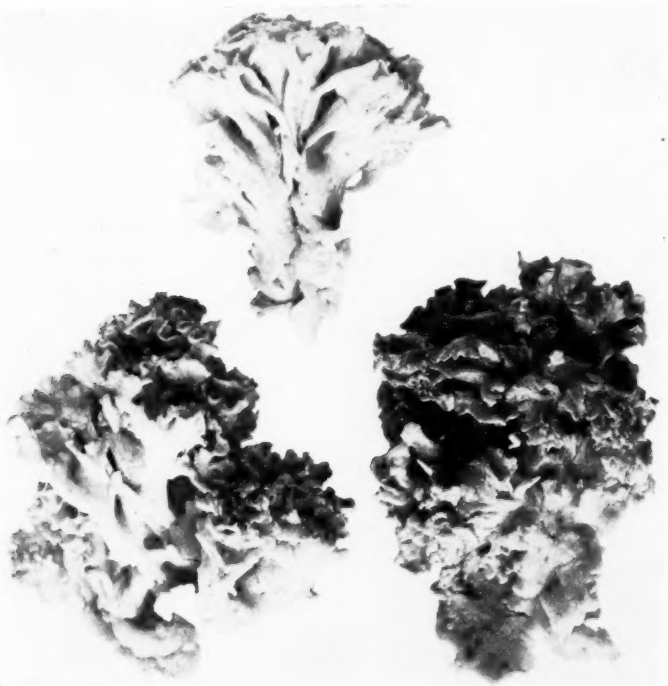

FIG. 1. SPARASSIS RADICATA

Small fruiting structures from a large sclerotioid stalk attached to the roots of *Pinus monticola*.

of the unilateral structure of the branches under various factors of growth and so forth is very much needed before the change should be accepted.

Lloyd has made the suggestion to the writer that the genus Sparassis falls naturally into two sections: first *S. crispa*, which is more of a fleshy

[4] Quélet. Flora mycologique de la France. Paris. 1888.

[5] Patouillard. Les Hyménomycètes d'Europe. Paris. 1887.

[6] Maire. Recherches cytologiques et taxonomiques sur les Basidomycètès. Annexé au Bull. Soc. myc. France. **18**:—. 1902.

[7] Lotsy. Vorträge über botanische Stammes geschichte. I. Jena. 1905.

nature especially near the base of the branches and is very closely related to Clavaria, and a second section consisting of species with thin lobes such as *S. spathulata* in the United States, *S. laminosa* of Europe, and *S. radicata* which has thinner lobes than either of the former. This seems to be a very logical arrangement. He further suggests[8] that *Sparassis laminosa* and *S. spathulata* are probably indentical. Their sporophores are certainly very similar.

THE ROOT STALK OF SPARASSIS RADICATA

Sparassis radicata (fig. 1) differs chiefly from *Sparassis crispa* (Wulf.) Fr. (*S. ramosa* Schäff.) which is reputed common in the eastern United States and in Europe, *S. laminosa* Fr. of Europe, and *S. spathulata* Schw. (*Stereum spathulatum* Schw.) (*sparassis Herbstii* Pk.) of America in the thinness of its lobes and by its very pronounced perennial sclerotioid rootstalk from which the sporophore develops annually (fig. 2). Since the rootstalk is usually attached to the deeper lateral roots of its host, it is often of a surprising length especially if a thick deposit of forest litter has accumulated around the base of the tree. Specimens have been found 50 cm. in length but the average is from 20 to 31 cm. No record exists of such a rootstalk for any other species. *Sparassis crispa* has a rooting base but it is not known to be perennial. It is possible that this phase of development is common to the other two species but has been overlooked. Sometimes the underground stalk is divided into two secondary ones each supporting a sporophore (fig. 2). The spongy character of the upper portion of the rootstalk soon merges into a very hard, compact mass and at the point of attachment to the root has very much the appearance of true cellular structure with the component filaments arranged longitudinally. The periphery of the stalk at the surface of the ground is composed of hyphae very much modified into a hard encrusting layer and may sometimes have a resinous appearance. The mycelium at the base of the stalk usually cements the earth into a hard stone-like body often of large dimensions. The fungus has not been found growing in the soil unattached to woody material. It is doubtful if it ever does so occur. All specimens so far collected were found at the base of trees.

The structure of the rootstalk is not that of a true sclerotium although it functions as such, is permanent and produces new sporophores from year to year. The stubs of old sporophores are plainly evident on the old root stalk (fig. 3) and as high as ten have been found on a single specimen. It was expected that the rootstalk would have great power of regeneration. This was tested on July 3, 1915 by cutting off a half-grown

[8] Lloyd, C. G. Letter No. 44, note 51, 1913.

sporophore. By August 30 a fully mature sporophore of average size had developed from the cut surface. On the former date also, a large root-stalk was severed from the roots of a Douglas fir, the fruiting end cut squarely off and the stalk buried loosely in moist forest soil. After a slightly longer time than that required for regeneration from the one attached to its host, a small fertile sporophore appeared from the normally

FIG. 2 FIG. 3

FIG. 2. SCLEROTIOID STALK OF SPARASSIS RADICATA

Showing lateral fruiting branch and tuberculate surface.

FIG. 3. UPPER PORTION OF SCLEROTIOID STALK OF SPARASSIS RADICATA

The remains of the sporophore of the season, two of the previous season, and two branches which produced sporophores in former years are shown. The fruiting nature of the latter was evident before the specimen was washed and dried.

fruiting end, showing not only the evident polarity[9] of the rootstalk but that it is a reserve structure of considerable reproductive power.

The formation of sclerotioid bodies from which their fructifications are developed is common to a number of Polypores. Chief among these noted in western United States are *Polyporus berkeleyi*,[10] *P. umbellatus*,[11] *P. frondosus*, and *Lentinus* sp., parasitic on the roots of conifers and probably unnamed. The latter species has a true sclerotium.[12] Lloyd[13] lists the following species growing from sclerotia-like structures and separates them as a distinct group of the section Ovinus of Polyporus: *Polyporus tuber-aster* (Japan, China, and Europe), *P. Goetzii* (Africa), *P. Sapurema* (Brazil), and *P. Mylittæ* (Australia). Three other species also with sclerotia but not included in this section are *P. basilapidiodes* (Australia), *P. sacer* (Africa), and *P. rhinocerotis* (Malay).

The formation of sclerotioid bodies seems to be common to the Clavariaceæ. Some of the large species of Clavaria are observed to spring from large globose masses which when sectioned exhibit a very compact structure and are known to last over for more than one year. This has been observed by the writer for *Clavaria aurea*, *C. amethystina* and *C. formosa*. The members of the interesting genus Typhula always, so far as observed, produce sclerotia from which the sporophore is produced. In view of the fact that the sclerotia-forming habit seems to be more or less common to the Clavariaceæ, together with the fleshy consistency of the sporophores, flattened or cylindrical anastomosing branches, large size of many species, amphigenous hymenium, constant in most genera, irregular in others, it seems that this family is very well defined. The removal of the genus Sparassis to the Thelephoraceæ, which possesses few or none of these characters, would be, it seems, an unnatural arrangement.

[9] Weir, James R. Untersuchungen über die Gattung Coprinus. Flora n.s. **103**: 301–305. 1911.

[10] Weir, James R. Some observations on Polyporus berkeleyi. Phytopath. **3**: 101–103, pl. 9. 1913.

Later observed by Lloyd, Letter No. 60, note 391, 1915; and Letter No. 59, note 306, 1915; and by Overholts, The Polyporaceæ of the Middle Western United States. Washington University Studies **3**: 23, pl. 2. 1915.

[11] Lloyd, C. G. Letter No. 58, note 277, 1915; and Overholts in The Polyporaceæ of the Middle Western United States. Washington University Studies. **3**: 24, pl. 2. 1915.

[12] Petch, T. The pseudo-sclerotia of Lentinus similis and L. infundibuliformis. Ann. Roy. Bot. Gard. Peradeniya **6**: 1–18, pl. 1. 1915.

[13] Lloyd, C. G. Synopsis of the Section Ovinus of Polyporus. 74–76. Oct. 1911. Cincinnati, Ohio.

THE DISEASE CAUSED BY SPARASSIS RADICATA

The observation that possibly some members of the genus Sparassis are parasitic on the roots of forest trees has been made by others. In a letter to the writer, dated January 7, 1916, Doctor Cotton writes: "*Sparassis crispa* has been found frequently, and from its intimate connection with the roots of Pinus and other conifers we are strongly inclined to suspect that it is parasitic."

Kirchmayr,[14] it appears, was the first to entertain the suspicion that Sparassis had symbiotic or parasitic tendencies. Working with *Sparassis crispa*, he found that the stalk of this species penetrated deep into the earth at the base of the tree (*Föhre*). Boring into the roots from which the fungus appeared to have sprung, he found that after passing through a zone of healthy wood the auger encountered diseased wood. This wood was of a brown color, gave out a strong odor of turpentine, and was very soft so that the auger readily pushed through it. Two trees when cut showed that the brown rot extended up into the heartwood of the trunk for a distance of two meters. The decayed wood could be rubbed into a fine powder and gave out an odor of turpentine. The decay resembled that produced by *Polyporus sulphureus*, the checks extending vertically and paralleling the annual rings. The checks were lined with a fine mycelial layer which was encrusted with granules of calcium oxalate. Large peices of the cubical checked wood could be removed from the hollow in the heartwood. The heartwood in the larger roots was also decayed, while the sapwood was infiltrated with pitch ("*verkient*"). The decayed wood largely dissolved in ammonia producing a thick brown liquid which on neutralization held a brown deposit in suspension.

The author was unable to demonstrate the relation of the mycelium of *Sparassis crispa* with that in the diseased wood. He calls attention to the fact that the shrinkage of the wood in the form of cubes with surfaces covered with a fine white mycelial layer, brown color, odor of turpentine, and ability to be rubbed into a fine powder are characteristic of the decay produced by *Polyporus schweinitzii*. In the writer's experience the rot of *Polyporus schweinitzii* may not always be accompanied by the production of sporophores until a long time after the wood is well advanced in decay. Since direct connection of the mycelium of the base of the sporophore with that of the decayed wood was not discernible, it seems quite probable that the investigator has made an incorrect diagnosis.

A careful examination by the writer of six trees, the roots of which bore the fructification of *Sparassis radicata* has not revealed, with one excep-

[14] Kirchmayr. Über den Parasitismus von Polyporus frondosus Fr. und Sparassis ramosa Schäff. Hedwigia **54**: 334–337. 1914.

tion, the presence of such decay as described by Kirchmayr. The exception was a case in which an old decayed sporophore of *Polyporus schweinitzii* was found buried in the litter at the base of the tree. The rot in a part of the root to which the Sparassis was attached showed the characteristic decay of *Polyporus schweinitzii* but could be easily distinguished from the decay produced by the former species. This strengthens the writer's assumption that the trees examined by Kirchmayr might possibly have been infected with *Polyporus schweinitzii* or *P. sulphureus*,

FIG. 4. FAN-SHAPED MYCELIUM OF SPARASSIS RADICATA IN THE BAST OF A ROOT OF DOUGLAS FIR

the rot of which is similar and is also mentioned by the author in connection with his description. It is possible that *Sparassis radicata* produces a different decay from that of *Sparassis crispa*. If this is true, it is at least an argument in favor of the former being specifically different from *Sparassis crispa*.

The great depth to which the rootstalk of *Sparassis radicata* penetrates the forest soil has made the investigation of the diseased roots difficult. When attached to the deepest lateral roots, a considerable excavation

was necessary before the point of attachment was revealed. In the case of two of the trees examined dynamite was used to expose the infected roots over a considerable area. This method was found serviceable since the thicker roots were broken up in such a manner as to expose the diseased wood and the mycelium beneath the bark to good advantage. In most cases the earth was carefully dug away from around the rootstalk leaving the entire fruiting body of the fungus standing free.

Upon removing the bark from any of the infected roots to which the fungus is attached, a yellowish white mycelium is exposed. This mycelium develops in the living bast and ramifies in all directions in fan-shaped layers (fig. 4). After the death of the root, small rhizmorphs develop from the border strands of the original mycelial layer. These

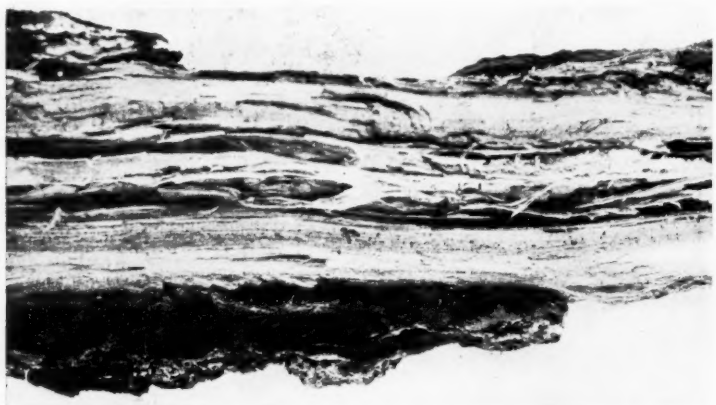

FIG. 5. TYPICAL DECAY OF HEARTWOOD CAUSED BY SPARASSIS RADICATA IN THE ROOT OF A DOUGLAS FIR

strands break up into mycelial filaments and penetrate the outer sapwood producing a brown or yellowish, laminated, carbonizing rot. The shrinkage of the diseased wood causes very small, narrow, elongated pits or shrunken areas to appear but they are not lined with mycelium. As is the case in some other rots, the initial stage of decay occurs principally in the region of the medullary rays which may become wholly disorganized before the surrounding tissues are affected. Very small brown rhizomorpha occasionally develop in the decayed wood either paralleling the tracheids or extending in zigzag lines. The early stage of decay is always preceded by a reddish color. A jet-black line sometimes marks the boundary of the decayed wood. The completely decayed root shrinks slightly away from the bark leaving a space which is eventually filled

with a thick, mycelial mat from which the rootstalk takes its origin. The greatest decay occurs at the point where the rootstalk is attached and is at first confined principally to the sapwood. Small roots originating from larger ones to which the rootstalk is attached are usually decayed throughout. The early decay of the heartwood in the larger roots is probably prevented by the large amount of pitch which they contain. Eventually the heartwood is invaded but is not broken down uniformly. Elongated pits filled with a white mycelium are formed in different parts of the wood, often anastomosing in such a manner as to leave long pieces of partially decayed or solid wood which may be very readily removed (fig. 5). Sunken areas on these pieces correspond to similar pits on pieces which have become wholly disorganized. These elongated pits are often bounded by a white mycelium arranged in the form of a network. The tissue in the heartwood is brittle but can not be rubbed into a fine powder as described by Kirchmayr for wood attacked by *Sparassis crispa*. The rot of the heartwood is always of a darker color than that of the sapwood. In Douglas fir it is brown; in spruce, of a more yellowish color. Away from the seat of first infection the mycelium may advance into the innermost heartwood causing the formation of a pitchy zone next to the sapwood. The diseased wood may be drawn out of such roots in strips leaving a hollow cylinder. The cambium and outer bast are always, however, permeated by the mycelium in the characteristic fan-shaped masses. The action of the mycelium in the resin ducts of the bast causes a flow of pitch which may cement the soil to the root in stone-like masses.

The fact that the fungus can maintain its activity in the cambium in roots deep in compact, mineral soil is very unusual. Some of the root fungi which attack primarily the heartwood may follow the roots to a considerable depth, and *Armillaria mellea* and *Fomes annosus* habitually attack the cambium to a considerable distance in the mineral soil, but in the experience of the writer no other species has developed this ability to as great an extent as *Sparassis radicata*. The decay is apparently confined to the roots proper, never having been traced beyond the surface of the soil. In case of an excessive accumulation of forest débris around the base of the tree the decay may extend higher up on the lateral roots than is ordinarily the case when this accumulation of materials does not occur.

Only two species of fungi are definitely known to parasitise the roots of coniferous trees in the temperate zone, viz, *Fomes annosus* and *Armillaria mellea*. *Rhizina inflata*[15] may possibly be grouped here but in northwestern United States seems to be confined principally to seedlings.

[15] Weir, James R. Observations on Rhizina inflata. Jour. Agr. Research **4**: 93-95. 1915.

There are a number of fungi which attack the roots of forest trees, are not strongly parasitic and do not cause a rapid browning of the foliage and rapid death. Their action is confined mainly to the heartwood of the roots and the base of the trunk. The most common of these is *Polyporus schweinitzii*. Other species which are either wholly confined to the roots and bases of trees or extend into the roots from infection through wounds on the trunk are *Trametes Pini*, *Echinodontium tinctorium*, *Polyporus sulphureus*, *Poria weirii*, and so forth. In the light of the present status of the study it can not be stated just how rapidly *Sparassis radicata* causes the death of its host. It has not been found on reproduction or young trees. The plant is not abundant but sufficient data have been assembled to show that it may be placed in the same group with *Armillaria mellea* and *Fomes annosus*.

To date only four trees, two Douglas firs (*Pseudotsuga taxifolia*), one white pine (*Pinus monticola*), and one spruce (*Picea engelmanni*) have been found to have succumbed to the action of the fungus. The conclusion that the death of these trees was caused by *Sparassis radicata* was arrived at because of the absence of any other fungus or factor which has heretofore been accredited as causing the death of trees. Several unhealthy trees with the fungus on their roots have been studied, but the common root fungi were present making a correct diagnosis impossible. The fungus has in every case, however, been found to cause the death of the living parts in the roots to which it was attached.

HOSTS AND DISTRIBUTION OF THE FUNGUS

Sparassis radicata is very widely distributed in the Northwest, having been found by the writer in British Columbia, Washington, Oregon, Idaho, and Montana. *Sparassis crispa* as reported from California is very probably based on this species.

The fungus has been found attacking the roots of the following conifers: *Pseudotsuga taxifolia*, *Picea engelmanni*, *Pinus monticola*, and *Larix occidentalis*. Its occurrence on the roots of broad-leaf species has not been noted by the writer. Kirchmayr cites instances of the occurrence of *Sparasssis crispa* on oak and beech and other broad-leaf species.

SUMMARY

The large species of Sparassis in the western United States is found to differ in a number of details from *Sparassis laminosa*, *S. crispa*, and *S. spathulata*, and is described as new under the name *Sparassis radicata*.

The fungus is chiefly distinguished by its thin lobes and an unusu-

ally large perennial rootstalk which is of the nature of a sclerotium and from which new sporophores are developed from year to year.

The most important feature in the life history of the species is its parasitism on the roots of conifers. The mycelium attacks the bast of the roots and later the wood, producing a yellow or brown, carbonizing rot.

OFFICE OF INVESTAGATIONS IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
MISSOULA, MONTANA

SOME CHANGES PRODUCED IN STRAWBERRY FRUITS BY RHIZOPUS NIGRICANS

NEIL E. STEVENS AND LON A. HAWKINS[1]

INTRODUCTION

The rot caused by *Rhizopus nigricans* Ehrenb. in strawberry fruits (*Fragaria* sp.) and potato tubers (*Solanum tuberosum*) has been studied by the writers. In both these cases as well as in others[2] the rot caused by this fungus is characterized by the rapid softening of the affected tissues accompanied by the loss of a large amount of juice. The morphological changes produced in the strawberry by *Rhizopus nigricans* have already been investigated. It was to obtain some information on the bio-chemical changes brought about in the strawberry fruit by this fungus that the present study was undertaken. In this work the effect of *Rhizopus nigricans* on the sugar, acid, pentosan, and crude fat content and the percentage of dry matter of the strawberry was studied.

METHODS

The berries used in this study were all of the variety Missionary, grown at Vienna, Virginia, and picked between May 20 and June 5, 1916. The methods for disinfecting and sampling used successfully by one of the writers in studies of peach (3) and potato (5) rots were found inapplicable to this work. The outer layers of cells of the strawberry were so injured by antiseptics, such as mercuric chlorid and alcohol, as to render results of doubtful value, especially as it had already been demonstrated that under normal moisture conditions the mycelium of the fungus grows chiefly in the outer cell layers (8). The texture of the strawberry, of course, prevents portions of the same berry being used for inoculation and control. The error due to variations in individual fruits may be somewhat greater than where portions of the same fruits can be compared.

[1] In the experiments described in this paper the cultural work was done by Stevens. The junior author is responsible for the chemical work. The writers are indebted to Mr. A. A. Riley of the office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations for assistance in the chemical work.

[2] The literature referring to the effect of *Rhizopus nigricans* on various fruits has been briefly reviewed in another paper soon to be published. Stevens, Neil E. and Wilcox, R. B., Rhizopus rot of strawberries in transit. U. S. D. A. Bul. 531.

After some preliminary experiments the following method was found satisfactory and was followed throughout the work: Berries as nearly uniform in size as possible were picked when nearly ripe, i.e., when about half of the berry showed a bright red color. They were picked early in the morning while still cool and covered with dew. The calyxes were removed and the fruit washed several times in sterilized, distilled water. They were then placed in wide-mouthed flasks which had been plugged with cotton and sterilized. Three or four berries were usually placed in each flask. The berries were inoculated with spores and mycelium from pure culture, a strain of *Rhizopus nigricans* isolated from strawberries shipped from Florida during February, 1916, being used for inoculation.

The method usually followed in preparing the samples of fruit for analysis was to grind the berries in a mortar and then wash the pulp quantitatively into the proper container. The flasks with the berries in them were weighed immediately before the fruit was prepared for analysis and the washed and dried flasks were weighed again after the berries and juice had been removed. The wet weight of the fruit could thus be calculated. All determinations were related to wet weight of the sound or rotten fruit. The methods for the determination of the sugars, pentosans, and dry matter were similar to those followed in the studies of peach brown-rot (3) and the rots of potato (6).

The acid content was determined by grinding a sample of fruit, usually about 20 grams, in a mortar, then allowing it to stand three days in a flask with 150 cc. water to which a little toluol had been added. The acid was titrated with n/10 sodium hydroxid in this flask using litmus solution as an indicator. The end-point in these titrations was not as exact as might be desired because the pigments of the strawberry which were present in the solution made it impossible to detect slight changes in color. However, the determinations are all comparative and the differences in acid content in the sound and rotten berries are large. The crude fat determinations were made by extracting the dried and ground samples of fruit with water-free ether, which was then evaporated and the residue dried and weighed.[2] A number of samples of freshly picked sound berries were analyzed to obtain some idea of the variation in the content of the compounds determined between the individual samples. The results of these analyses are shown in table 1.

From table 1 it may be seen that there is some variation in the content of the compounds determined, especially the acids and sugars. The

[2] Wiley, H. W., ed. Official and provisional methods of analysis. Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted, 1912.

TABLE 1

Showing the content of sugars, pentosans, acids, crude fats, and dry matter in freshly picked strawberries. Three separate samples used in the determination of each substance

PENTOSANS AS PERCENTAGE WET WEIGHT	ACID AS CC. NORMAL ACID PER 100 GRAMS WET WEIGHT	REDUCING SUGARS AS PERCENTAGE WET WEIGHT	SUCROSE AS PERCENTAGE WET WEIGHT	CRUDE FATS AS PERCENTAGE WET WEIGHT	DRY MATTER AS PERCENTAGE WET WEIGHT
0.57	2.59	2.89	1.37	0.27	7.97
0.59	2.10	3.67	1.69	0.29	8.17
0.56	2.15	4.03	1.65	0.34	8.15

variation in pentosan content is slight. There is a considerable amount of sugar and the acid content is rather high. The berries of course were not ripe when picked and analyzed but were at about the stage of maturity at which they are harvested in some sections of the South where this variety is grown commercially. A comparison of the results of these determinations and the data obtained from the analysis of the sound strawberries which were analyzed three, seven, or fourteen days after harvesting gives some data on the changes which take place in picked strawberries during ripening.

In table 2 is shown the pentosan, acid, sugar, and crude fat content and the percentage of total dry matter in the sound and rotted samples of fruit at different times after harvesting. The analyses are also of some interest in showing the amount of the various substances in the strawberry fruit. Considerable work has, of course, already been done on this subject by various investigators. A review of much of this work is given by Wehmer (9, p. 284–285). In table 2 the results given are averages of at least three determinations of the various compounds on as many separate samples.

TABLE 2

Comparative pentosan, acid, sugar, and crude fat content and the amount of total dry matter in sound and rotten strawberries

NUMBER OF DAYS AFTER INOCULATION	PENTOSANS AS PERCENTAGE WET WEIGHT		ACID CONTENT AS CC. NORMAL ACID, PER 100 GMS. OF FRUIT		REDUCING SUGARS AS PERCENTAGE WET WEIGHT		SUCROSE AS PERCENTAGE WET WEIGHT		CRUDE FAT CONTENT AS PERCENTAGE WET WEIGHT		DRY MATTER IN STRAWBERRY AS PERCENTAGE WET WEIGHT	
	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit
3	0.51	0.51	1.98	1.89	1.95	1.35	1.89	0.66				
7			0.76	1.49	0.44	0.13	0.20	0	0.36	0.40	7.19	6.19
14	0.38	0.32	0.73	1.55					0.34	0.31	6.63	5.96

From the results shown in table 2 it seems that the pentosan content is no lower in the rotten fruit three days after inoculation than in the corresponding sound samples. There is, however, a decrease in the pentosans as calculated on a wet-weight basis after the fungus has acted fourteen days. It seems probable then that the fungus utilized a portion of the pentosans. It is interesting to note that there is a somewhat similar decrease in the percentage of dry weight in the inoculated berries in two weeks so that if the pentosans were calculated on the basis of dry weight at the time of analysis the percentage of the furfurol yielding substances in the sound and rotten fruit would be approximately the same.

The effect of the fungus upon the acids seems to be to reduce the acids slightly, as the acid content after the first three days is somewhat lower in the rotted samples than in the corresponding sound ones. The acid content of the sound berries decreased rapidly until at the end of seven days it was only about half that of the rotted berries, and a similar ratio is evident seven days later. From a comparison of the acid content of the sound berries in table 2 with that of the freshly picked berries shown in table 1 it is evident that there is a gradual decrease in the acidity the longer the berries are allowed to stand and that this decrease is much more rapid in the first week. That this decrease was not due to a neutralization of the acid by ammonia either in the sound or rotten fruit was shown by the negative results obtained from several series of ammonia determinations by Folin's method. The acid is apparently used up by the berry in its metabolism, probably in respiration. That the decrease in acidity in the sound fruit was greater than that in the rotted berries seems to indicate that the mechanism for the utilization of this acid is destroyed or its action inhibited by the fungus. The fungus apparently uses little of the acid.

The sugar content of the rotted berries is always lower than that of the sound fruit of the same series of samples. A comparison of the percentage of sugars in the sound fruit, as given in tables 1 and 2, shows that the sugar content rapidly decreases after the strawberry is harvested. The sugars as well as the acids are apparently used by the strawberries in respiration or in other metabolic processes. The much more rapid decrease in sugar content of the inoculated fruit is evidence that the fungus uses the sugars.

The percentage of ether soluble material in the sound and rotted berries, considered in the tables as crude fats, does not undergo any decided decrease when the berry is rotted.

The percentage of dry matter in the rotted berries is less than in the sound fruit.

As has been mentioned above, this strawberry rot is characterized by

a rapid softening of the tissue, the loss of water, and apparently a general collapse of the berry. It was considered of interest in this connection to determine the amount of sugar and acid present in the juice which escapes from the inoculated berries as compared to that in the juice of healthy berries picked at the same time and maintained under the same conditions of moisture and temperature. For this experiment the two samples of berries were picked, washed in sterilized water, and placed in liter flasks. The berries in the one flask were inoculated with Rhizopus in the usual way, while those in the other flask were maintained as controls. The flasks were filled to the same height and were allowed to stand in the laboratory for three days. The juice was then poured off the inoculated fruit and the berries of the control sample were frozen with carbon dioxid, and the juice expressed with a fruit press. The sugar and the acid content of the samples of juice from both lots of berries were determined according to the usual method. The results are shown in table 3.

TABLE 3

Sugar and acid content of juice from sound and rotted strawberries

SUGAR (PERCENTAGE)				ACIDITY IN TERMS OF NORMAL ACID	
Juice from sound fruit		Juice from rotted fruit		Juice from sound fruit	Juice from rotted fruit
Reducing sugar	Sucrose	Reducing sugar	Sucrose		
2.66	2.98	0.62	0.14	0.191	0.208

	JUICE FROM SOUND FRUIT	JUICE FROM ROTTED FRUIT
Δ	0.694	1.037
Diffusion tension in atmospheres	8.37	12.50

From table 3 it is apparent that the acidity of the juice from the diseased berries is slightly higher than that of the juice from the sound ones, while the sugar content is considerably lower. In table 2 it is shown that the sugar content of the diseased fruit three days after inoculation was decreased considerably below that of the control samples. This may, of course, account for part of the difference. However, the sugar content of the inoculated berries after three days is a little over half that of the sound fruit, while the sugar content of the juice that leaks out of the rotted fruit is about one-eighth that of the juice expressed from sound berries. The utilization of the sugar by the fungus, then, can hardly account entirely for the lower sugar content of the juice from the inoculated berries and much of the sugar must still remain in the infected fruit.

Other materials than sugars and acids are, of course, present in the strawberry juice. In order to obtain some idea of the amount of substance in this watery extract, freezing point determinations were made on the two samples of juice. These determinations were made with a Beckmann freezing point apparatus in the usual way. The depression of the freezing point (Δ) of these juices below that of distilled water and the calculated diffusion tension (7, p. 30–31) or osmotic pressure of which these juices are capable are shown below:

The freezing point of the juice from the rotted berries is considerably lower than that of the juice from the sound fruit. As calculated by this method the solution from the rotted berries has, obviously, a higher diffusion tension than that from the sound fruit. The juice from the rotted berries then is a more concentrated solution of some substance or substances than is the juice from the sound fruit. From these experiments with the juice from the sound and rotted fruit it is evident that the juice which escapes from the rotted berries contains at least a part of the soluble matter that is present in the cell sap of the berry before it is attacked by the fungus.

DISCUSSION

The effect of the fungus upon the various constituents of the strawberry as shown in the foregoing pages is much the same as has been shown for other fungi and other host plants in similar studies. Most fungi apparently utilize the sugars in their hosts when growing parasitically. This has been shown by one of the writers in the case of the brown-rot disease of the peach (3) and some of the Fusarium rots of potatoes (6).

That the fungi sometimes lower the pentosan content of their host when living parasitically has also been shown (4, 6). *Rhizopus nigricans* apparently does not utilize the acids of the strawberry to any extent. Some fungi are apparently able to use the acids in their host plant while others are not, probably depending on the ability of the fungus to assimilate the specific organic acids that are present in the host. Behrens (1, p. 700–706) has shown that the acids in apples can be used by fungi. Sclerotinia, however, apparently had little effect on the acids in the peach fruit (3).

In considering these results it should be remembered that at the time of the first analysis, i.e., three days from the time of inoculation, leak had progressed to an advanced stage. That is, the berries were flattened and a large amount of juice had escaped. At this time, as shown by table 2, only relatively slight changes have taken place in the amounts of the various constituents for which analysis was made. There is apparently no difference in the pentosan content between the rotted berries and

sound berries of the same age, and the difference in the amount of acid present is very slight. Some reduction in the amount of sugar, both sucrose and reducing sugar, has of course occurred but as the sugar was probably chiefly contained in the cell sap this change offers no clue as to the cause of leaking.

The bio-chemical studies have, then, served to confirm the conclusion derived frum the histological study that the changes, detectable by the methods followed, which have taken place in the cells of the strawberry at the time leak occurs are relatively slight. The histological study showed that the cell walls of the strawberry are seldom pierced by the fungous hyphae and that the protoplasm of the cells is only slightly altered in appearance, the nuclei in particular retaining their normal appearance until the cells are crushed.

In accounting for the loss of juice which occurs in strawberries attacked by *Rhizopus nigricans* the only tenable hypothesis seems to be that the fungus so affects the protoplasm of the cells, perhaps by secreting some toxin, that it is no longer capable of functioning as a semi-permeable membrane. In this connection it is interesting to note that Gortener and Blakeslee (2) have recently demonstrated the presence of a substance in Rhizopus which is extremely toxic to rabbits. Whether the protoplasm of the strawberry is killed at once by the fungus or whether it is anesthetized and rendered permeable to the material dissolved in the cell sap is an open question. Further investigations on this subject are planned.

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WITCHES-BROOMS ON HICKORY TREES

F. C. Stewart

With One Figure in the Text

In Ontario county, New York, trees of the shell-bark hickory, *Carya ovata*, occasionally bear witches-brooms apparently caused by the fungus *Microstroma juglandis* (Bereng.) Sacc. During winter, while the trees are bare, the "brooms" are readily detected at a considerable distance. They are typical witches-brooms consisting of compact clusters of short, upright branches. They are of all sizes up to about two-thirds of a meter in diameter in the bare state and, of course, considerably larger when in foliage. As many as thirty "brooms" of various sizes have been observed on a single large tree.

The leaves on the "brooms" are yellowish green above, and white and mealy with Microstroma spores on the under surface. Usually, they are smaller than normal and much curled. In mid-summer they blacken on the margins, then wither and fall prematurely. The fallen leaves are not replaced by new ones as happens with the cherry witches-brooms caused by *Exoascus cerasi*. The branch bearing the "broom" is, usually, considerably enlarged at the point of attachment of the "broom" and often dead beyond the point of attachment.

The constant occurrence of *Microstroma juglandis* on the leaves leads to the belief that this fungus is the cause of the "brooms." Almost every leaf on every "broom" shows the fungus over its entire under surface while the leaves on all other parts of the tree may be wholly free from Microstroma. The presence of the fungus becomes evident as soon as the leaves unfold in the spring. This condition of affairs is not rare. It has been observed during seven consecutive seasons on a large number of "brooms" on nine separate trees in three localities—Geneva, Canandaigua and Victor.

On the other hand, *Microstroma juglandis* has long been known as a parasite on the leaves of walnut and hickory and is widely distributed in Europe and America; yet its association with witches-brooms has not been previously recorded. In fact, the writer has been unable to find any published account of witches-brooms on hickory trees. The writer, himself, has occasionally observed *M. juglandis* on the leaves of hickory trees which bore no witches-brooms. At Geneva, in 1916, this was of com-

mon occurrence. In such cases the fungus appeared on the under surface of yellowish spots or small areas instead of covering the entire lower surface of the leaf. This fungus was morphologically indistinguishable from that occurring on the leaves of the "brooms."

In only a single instance has the writer had an opportunity to study a "broom" in its incipiency. This was a "broom" discovered on June 10, 1914. It consisted of a single new shoot growing vertically from a

FIG. 1. WITCHES-BROOMS ON A HICKORY TREE, CARYA OVATA

This tree bore seventeen "brooms" the largest of which was nearly a meter in diameter.

small enlargement about 13 cm. back of the tip of a branch 1 cm. in diameter. On the parent branch beyond the point of attachment of the "broom" the bark was stil green, but there were no leaves and the terminal bud was dead. Apparently, the infection had occurred in 1911. The "broom" arose from wood three years old.

The new shoot constituting the incipient "broom" bore seven leaves, three of which were free from fungus, while on each of the other four some

of the leaflets were attacked by Microstroma. On an adjacent branch there was a larger "broom" every leaf of which was covered with Microstroma, but none of the other leaves on the tree were affected. A later examination, made on July 2, revealed no change in the "broom" except that the margins of the affected leaflets had begun to blacken. Further observations were impossible owing to the accidental destruction of the young "broom."

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A NEW LEAF-SPOT DISEASE OF CHERRIES

BERT A. RUDOLPH

WITH THREE FIGURES IN THE TEXT

In August, 1913, a conspicuous leaf-spot disease of sweet cherries was observed by the writer near San Jose, California, and specimens have been received from the principal cherry growing sections between Redlands, California, and Corvallis, Oregon. No mention of it has been found in any of the literature examined. Descriptions were sent to F. C. Stewart, Geneva, New York; Howard S. Reed, Blacksburg, Virginia; M. B. Waite, Washington, D. C., and Donald Reddick, Ithaca, New York, and all expressed doubt as to its occurrence in their localities. It is believed to be peculiar to the Pacific coast.

Dead, definite, circular spots from one to about 14 mm. in diameter appear on the leaves. The dead areas are a pronounced reddish brown or chestnut to mahogany color, and are sharply differentiated from the living tissue, extending from epidermis to epidermis and commonly marked with a delicate, concentric zonation consisting of narrow lines and darker bands. Several spots may coalesce to form one large one.

On the upper surface of the leaf may nearly always be found a minute, whitish gray pustule located in the center of the spot, and about which the zones are concentric. The pustules are uplifted bits of epidermal leaf tissue, and a minute insect of the family Chalcididae has regularly been found in them.

Within the spot there may be a definite, central, circular portion which is lighter than the remainder. This inner area sometimes reaches 6 mm. in diameter and is ochraceus to ferrugineus (Saccardo's Chromotaxia), or it may be entirely absent. The darker portion is castaneus to badius. The pustule, if present, is located in the center of the inner, lighter area, if such an area occurs. The under side of the spot presents a slightly different color from the upper. The inner lighter area is isabellinus, and the remaining portion latericius, being lighter than the corresponding area on the upper surface.

The dead tissue remains intact within the leaf.

Sometimes the spots spread out in an irregular, somewhat indefinite, mosaic-like fashion (fig. 1).

The spots just described are produced by a fungus of the genus Alternaria which usually gains entrance to the leaf tissue through the injuries made by insects which produce the pustules. Cultures of the causal organism were obtained as follows: The leaves were first lightly sponged with alcohol. Bits of the spots were removed with a flamed scalpel and planted in plates of non-nutrient agar. From these plantings an Alternaria and

FIG. 1. SPOTS PRODUCED BY ALTERNARIA CITRI PIERCE VAR. CERASI ON CHERRY LEAVES

At the apex of one leaf is a mosaic-like spot produced by this organism. Insect pustules by which infection took place are seen in the center of the spots.

three other distinct fungi developed in more or less homogeneous colonies. Plantings were taken from these and placed in individual plates of non-nutrient agar and a homogeneous culture was obtained of each fungus. On October 16, 1913 these fungi were inoculated into young leaves of seedling cherries in the conservatory of the University of California.

Four leaves on one tree were inoculated, each with a specific fungus.

The leaves were first lightly sponged with alcohol. Bits of agar, bearing spores and mycelium, were taken with a flamed needle and placed on the surfaces of the leaves, and the needle was pushed through the bits of agar into the leaf tissue. The tree was then sprinkled with tap water and covered with a bell-glass. Within four days four large spots were produced on the leaf which had been inoculated with the Alternaria. The spots were more or less circular with diameters of about 7 mm. and were characterized by being of a wilted appearance, and of a greenish-brown color, and having three or four light-colored zones. The bell-

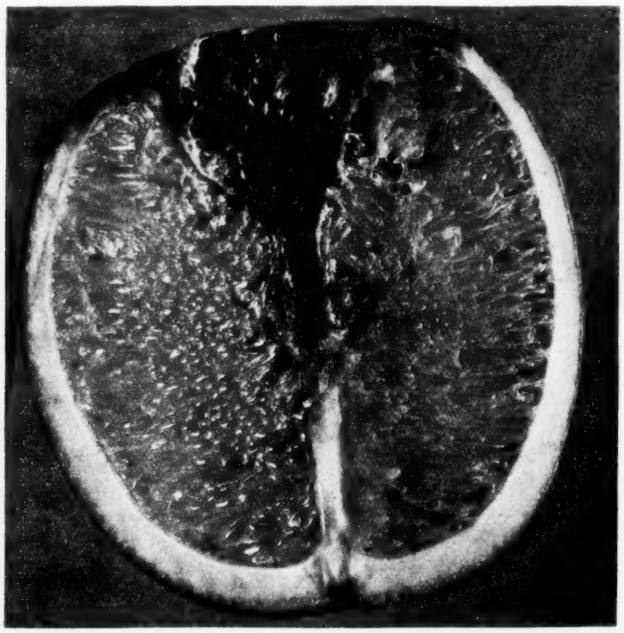

FIG. 2. BLACK HEART PRODUCED IN A NAVEL ORANGE BY INOCULATION WITH ALTERNARIA CITRI VAR. CERASI

glass was removed, and the killed areas grew slightly larger and lighter in color as they dried out. The inoculations with the three other fungi gave negative results. About twelve inoculations were made with each of them soon after on healthy cherry leaves, taking care to prick the tissue in each case, but the results were negative. Bits of the artificially produced spots were planted in nutrient agar, and a copious growth of but one fungus—the Alternaria-developed. A pure culture of this strain was then secured by the single-spore method and was used in all succeeding inoculations.

The details of subsequent inoculations are recorded in the writer's thesis, deposited in the library of the University of California. In all, over two hundred inoculations were made on the following varieties of cherries; Royal Ann, Richmond, Morello, Black Tartarian, seedling sweet cherry and California Wild Cherry (*Prunus ilicifolia* Walp.). During the winters of 1913 and 1914 experiments were confined to the leaves of seedlings. In the spring of 1914, however, large numbers of inoculations were made upon the various named varieties. The method of inoculation was the same as that described earlier. Checks were made on one side of the mid-rib and inoculations with a flamed needle on the opposite. Not more than ten of all the inoculations made in this manner were negative. In most of the inoculations no bell-glasses were used, and the spots developed quickly, although less rapidly than where covered. About twenty other inoculations were made in lots of two to five by laying bits of the fungus in agar on the leaves without puncturing or injuring them in any manner. All these were negative. The greater part of this work was done in a greenhouse without heat.

The fungus is particularly active when inoculated in the leaves of California Wild Cherry. About three dozen inoculations were made on this plant, and not more than five proved unsuccessful. Frequently artificially inoculated leaves were so badly affected as to be shed from the tree. This was especially common when the trees were kept under bell-glasses. The spots produced differ greatly in color from those on the leaves of sweet cherries. There is usually a circular, inner area in each spot which is avellaneus on the upper side and isabellinus on the lower. The remaining or outer portion of the spot is isabellinus, and the lower side latericius. At times the spots may be a deep brown color, especially when formed more slowly.

Microtome sections made of the freshly produced spots stained with congo red and methylene blue show best the action of the fungus. The parasite is intercellular. The chloroplasts of the cells lying just beyond the tips of the advancing mycelium are first affected and cannot be distinguished. The cells collapse and disintegrate rapidly as the fungous threads come in contact with them.

All inoculations in the bark and wood of normal cherry twigs were negative. Over two dozen inoculations were made on stems up to 2.5 cm. in diameter. The bark was first sponged with alcohol. Slant cuts were made with a flamed scalpel and the infectious material placed beneath the flap, or inoculations were made by puncturing the bark through the spore-bearing material. Both types of inoculations were either left exposed or wrapped with thoroughly boiled linen strips or bound in absorbent cotton. Checks were also made in the same way. The wounds healed normally, the plants apparently being unaffected by the fungus.

When inoculated in the leaves of other plants the results are often as pronounced as in the cherry leaves. The leaves were first sponged with alcohol and the inoculations and checks were made in the usual manner. The results obtained are shown in table 1.

TABLE 1

Results of inoculations of leaves of various hosts with a species of Alternaria from cherry leaves

HOST	NUMBER OF INOCULATIONS	RESULTS	OBSERVATIONS
Apple (Pyrus Malus L.)			
Winesap	20	All positive	Large reddish spots
Newtown Pippin	25	All positive	Large reddish spots
Box Elder (Acer negundo L.)	50	All positive	Large brown spots
Hungarian prune (Prunus domestica L.)	18	All positive	Small brown spots. Developed slowly
Wickson plum (Prunus triflora Roxb.), (P. Simonii Carr.) hybrid	26	All positive	Small brown spots. Developed slowly
Orange (Citrus nobilis Lour.) King Mandarin	20	Doubtful	Spots barely larger than on the checks
Loquat (Eriobotrya japonica Lindl.)	21	Negative	Spots no larger than on the checks
Potato (Solanum tuberosum L.)	30	Negative	Spots no larger than on the checks
Avocado (Persea gratissima Gaertn.)	32	All positive	Large reddish brown spots
Watermelon (Citrullus vulgaris Schrad.)	16	All positive	Black spots. Developed slowly
Peach (Prunus persica S. & Z.) Crawford peach	40	Positive	Irregular gray-brown spots

In general it was found that the fungus produced its optimum growth in the leaf tissue when the atmosphere was moist and warm and sunlight at a minimum. The mere shading of an infected leaf with a piece of paper was found to permit the production of larger spots in a shorter time than where the leaves were exposed to direct sunlight. The fungus is a typical wound parasite, all inoculations on uninjured leaves having failed. When a young leaf was inoculated before being fully developed a shot-hole effect sometimes resulted on its expansion.

The fungus grew vigorously on the common culture media, and its more important characteristics are as follows:

On non-nutrient agar. Growth rapid, mostly confined to the surface of the medium which is not discolored. A small amount of long, aerial, dry, silky, gray-white mycelium is produced. Spores thinly scattered over the surface of the medium.

On nutrient agar (containing meat extract, peptone and salt.) Growth vigorous, a copious aerial, downy mycelium is developed consisting of long, branched, silky, gray hyphae. Spores commonly produced in greatest numbers in concentric zones which are dark green at first becoming sooty black with age. The spores are olivaceus under the microscope. The agar is cleared of any cloudiness by the fungus as it develops.

On steamed rice. Growth vigorous. A snow-white, aerial, downy mycelium first develops which darkens to a dirty, greenish gray with age. A flesh-colored pellicle is produced upon the surface of the medium darkening with age to black. The rice grains gradually become colored a light yellow. Spores are produced close to the surface of the pellicle and beneath the longer aerial hyphae. They are pale olivaceus under the microscope.

On bread and prune juice (Duggar's Fungous Diseases of Plants, p. 24). Growth vigorous. A copious, downy, aerial mycelium is produced which is dull white at first becoming a dirty greenish gray with age. Patches of older parts of the aerial mycelium are often yellowish. A cream colored pellicle is formed on the surface of the medium becoming black with age. Spores develop close to the pellicle beneath the longer, aerial hyphae. They are dark olivaceus under the microscope. The medium becomes darker as the fungus develops upon it.

On steamed potato slants. Growth vigorous. A white, downy, aerial mycelium is first produced becoming a dirty greenish gray with age. A pellicle is formed upon the surface of the slant and may be flesh colored or greenish, becoming black with age. Finally the aerial mycelium usually collapses, and only a black, shining pellicle is observed. After growth has entirely ceased the plugs no longer react for starch with iodine but give a good test for reducing sugar with Fehling's solution.

On beet agar. Growth vigorous. An aerial, downy mycelium is first produced which is gray-white becoming greenish and finally black with age. Cultures have a sooty, granular surface punctuated with whitish hyphae in scanty tufts and occurring singly. The aerial mycelium may or may not be somewhat zonate. Spores are inclined to be smaller and decidedly darker than those found on other media, being olivaceus to fuligineus under the microscope.

On steamed cherry twigs. Growth vigorous. A copious, downy, aerial, white mycelium is first produced which becomes a dirty greenish gray to black with age, giving the cut surfaces a sooty appearance. The bark

is sparsely covered. Spores are produced in abundance close to the cut surfaces of the twigs. They are somewhat smaller than those on various other media and are dark olivaceus to fuligineus under the microscope.

On navel oranges (Sterilized by washing the surface with mercuric chloride solution). When inoculated in moist chambers at the navel end a black rot of the rag or pulp cells results which is identical with that produced by *Alternaria Citri* Pierce (fig. 2). At the point of inoculation an aerial mycelium develops which is pulvinate and gray-white at first, becoming a dirty greenish gray with age. The rind discolors becoming olivaceus in a gradually increasing area around the fungus colony. Several months after inoculation the aerial mycelium, having overrun the orange, bleaches out and ultimately becomes a beautiful pink. The whole fruit gradually settles down with a soft, moist rot.

On + 5 nutrient agar agar. This medium remains liquid due to the high acid content. The isolated colonies of aerial mycelium are whitish at first becoming sooty black with age and rounded or hemispherical. On titrating the medium three weeks after planting it was found to have been reduced to + 4. The color was changed from a light amber to a deep brown (fuligineus). This destruction of acid by the fungus was observed on various other media. The average of four titrations was always taken.

In general it was observed that the color, shape and size of the spores produced on various media may vary slightly, but the most important characteristics remain the same.

The fungus bears a striking similarity to *Alternaria Citri* Pierce and even a closer relationship to an Alternaria found on watermelon leaves. Pure cultures of the three fungi were obtained by the single-spore method. In drop cultures of +2 nutrient broth the fungi may be said to be identical morphologically. Possibly the spores of *A. Citri* are slightly rougher than those of the other two, but this difference was not found to be constant. The three fungi cannot be differentiated on nutrient and non-nutrient agar, and the rots produced by them in navel oranges are identical.

The cherry Alternaria cannot be distinguished from the watermelon Alternaria on +5 nutrient agar, but is distinguished from *A. Citri* on this medium. The latter produces colonies which are circular, whitish at first, becoming gray with age. They are also flat or depressed with crater-like centers which are darker in color (griseus-olivaceus).

The cherry Alternaria is distinguished from the other two Alternarias when inoculated on cherry leaves only by the size of the spots and the rapidity with which they are produced. The watermelon Alternaria produces the smallest spots, and they develop more slowly, but the difference is very slight. Over four dozen inoculations were made with *A. Citri* and the watermelon Alternaria.

The cherry Alternaria was distinguished from the other two by its action on watermelon leaves (var. Cuban Queen), being the least virulent of the three fungi. Sixteen inoculations each were made with the cherry Alternaria and *A. Citri* on separate plants. Twenty-four inoculations were made with the watermelon Alternaria on a third plant. The black spots produced were identical, but the watermelon Alternaria spread to the stem, killing the plant, while the cherry Alternaria confined itself to the leaves. *A. Citri* formed slightly larger spots than the cherry Alternaria but also confined itself to the leaves.

The cherry Alternaria may be further distinguished from the other two in the matter of spore germination. Fresh spores of the cherry Alter-

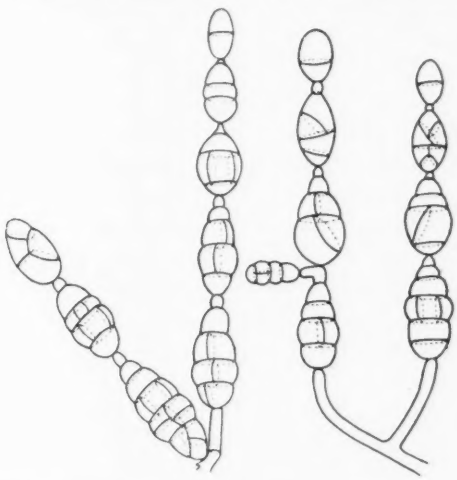

FIG. 3. SPOROPHORES AND SPORES OF ALTERNARIA CITRI VAR. CERASI

From drop culture of + 2 beef broth with peptone. × 500

naria will germinate in neutral, +1, and +2 broth in less than twenty-four hours, but in +7, and +8 broth germination rarely takes place in less than a week. It will grow and produce spores in +8 nutrient broth. Spores of the watermelon Alternaria will germinate in +8 broth and grow feebly, but the fungus has rarely been observed to produce spores in this medium. Spores of neither will germinate in +9 broth. *A. Citri* will grow and produce spores in +6 broth, but spores will not germinate in +7 broth. Spores of both the cherry and watermelon Alternarias germinate in +7 and +8 broth with the greatest difficulty, the germ-tubes developing in an abnormal manner and resemble the budding of yeast. Ordinarily the germ tubes are straight with but few septa, but in broths of high acidity, the opposite is the rule.

The cherry Alternaria cannot be distinguished from the others by germination tests in alkali solutions. All germinate readily in tap water containing 8 per cent normal alkali (sodium hydroxid), but in −10 tap water germination may not take place for a week. None of the three fungi will germinate in −11 tap water.

On steamed rice the watermelon Alternaria colors the rice grains a slightly lighter shade of yellow than does the cherry Alternaria, while *A. Citri* colors them all shades of yellow to latericious. In other respects the fungi are identical on this medium.

On +5 nutrient agar the growth and acid destruction by the cherry Alternaria was identical with that of the watermelon Alternaria. *A Citri* within the same time only reduced the acid content 0.5 per cent instead of 1 per cent.

The longevity of the cherry Alternaria is largely dependent upon the presence of moisture. However, spores over fourteen months old produced on steamed cherry twigs, which dried out soon after the planting of the fungus on them, were found to be viable, although germination rarely took place in less than a week in neutral or +1 broth.

The various experiments enumerated show the three fungi to be very closely related. The name of the watermelon Alternaria is not known, but it is not believed to be *A. cucurbitæ* Let. which is also parasitic on melon vines. The spores of *A. cucurbitæ* are described as longer and narrower—mostly 60 to 68 by 8 to 9 μ—while the spores of this particular Alternaria are only 10 to 47 by 6.8 to 15μ.

The cherry Alternaria is not believed to be the same as *Alternaria Cerasi* Potebnia found at Kharkov, Russia, the spores of that fungus being decidedly larger and produced in velvety patches on dry margins of leaves, according to Saccardo.

Believing this fungus to be hitherto undescribed, and being of the opinion that its close relationship to *A. Citri* Pierce entitles it to be classified as a variety of that species the writer suggests the name *Alternaria Citri* Pierce, variety *Cerasi* with the following technical description.

Alternaria Citri Pierce var. **Cerasi** nov. var.

Producing dead spots on leaves of sweet cherry. Spots 2 to 14 mm. in diameter, reddish brown or chestnut above and lighter below, often faintly zonate, sometimes with a distinct, lighter colored central area and usually starting from an insect injury, sometimes extending outward in an indefinite mosaic.

A wound parasite only, as shown by artificial infections, capable of producing spots on leaves of numerous plants. Distinguished from *A. Citri* Pierce with difficulty.

Mycelium, in leaves of sweet cherries, sub-epidermal, of slender, septate, hyaline hyphae, 1 to 3 μ in diameter. Aerial mycelium very rarely produced and then only in the presence of unusual humidity and optimum temperature, hyphae 3.4 to 4.8 μ wide, gray-white to pale olivaceus, long, silky, branched, septate; conidia, not observed in field, occasionally produced in presence of unusual humidity and optimum temperature—then close to the lower surface of the leaf, never on long, aerial hyphae, clavate fusiform or elliptical at maturity, 15.3 to 57.8 by 6.8 to 15.3 μ, muriform, translucent, olivaceus-brown, slightly verrucose or smooth, becoming constricted at the septa with age, transverse septa commonly parallel, 3.3 to 9.9 μ apart, with short, smooth, hyaline-subhyaline isthmus which is usually 3.4 by 3.4 μ; several catenulate, in cultures 2 to 7 in simple or branched series, somewhat variable in size, form and color, germination from any cell, produced abundantly on various culture media. Conidiophores, short, in cultures 1.7 to 153 μ by 1.7 to 6.8 μ, olivaceus-subhyaline, erect, in general narrower than the vegetative hyphae.

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BLISTER SPOT OF APPLES AND ITS RELATION TO A DISEASE OF APPLE BARK

DEAN H. ROSE[1]

WITH THREE FIGURES IN THE TEXT

In the present paper is described a disease of apples (fruits) which has been under investigation through two growing seasons. A brief report has already been published by the writer[2] but so far no other mention of it has been found in the literature. There is given also a description of a disease of apple bark which seems to be causally related to the fruit disease. The experimental proof is not yet complete but considerable evidence that the relation actually exists has been obtained and will be found summarized in the second part of the paper.

BLISTER SPOT OF APPLES

Occurrence and general appearance of the disease

The blister-spot disease was first noticed on July 6, 1915, on Norfolk Beauty (dwarf) as roughly circular or sometimes irregularly lobed shallow blisters, varying in color from light brown to black, and in size from 1 to 5 mm. in diameter (average about 2 mm.) by 0.2 mm. in depth. Search through the experiment station orchard at Mountain Grove, Missouri, then showed similar spots on Melon, Ishewold, and Hawley. In 1916 spots were first found about the middle of June on Blue Pearmain, Higginbotham, Yellow Transparent, Benoni, Melon, Rock Pippin, Lansingburg, Early Ripe, Victuals and Drink, Isham, Ishewold, Hawley, Norfolk Beauty, Red Astrachan, White Pippin (wrongly given as Yellow Newton in the report mentioned earlier),[2] Klondyke, Duling, and Jonathan. Allowing for the fact that such varieties as Yellow Transparent and Red Astrachan were gone when affected apples were discovered in 1915, this list shows the disease much more prevalent in the station orchard in 1916 than in 1915. Little is known of its distribution. Apples showing typi-

[1] The writer wishes to acknowledge his indebtedness to Mr. Harold Swartout and Miss Beatrice White, without whose careful and efficient help the work here reported could not have been accomplished in the time available.

[2] Rose, Dean H. Blister spot of apples (abstract). Phytopath. **6**: 110. Feb. 1916.

cal spots, variety unknown, were received in May, 1916, from Marionville, Missouri, and what looked like an early stage of the disease was observed in 1915 on Ben Davis at West Plains, Missouri. No cultures were made from these apples.

Lesions occur at the lenticels and usually on the lower half of the apple. Benoni and Jonathan show spots only around the blossom end, while Higginbotham, Melon and others may be affected over any part of the surface except the upper one-fifth (fig. 1, *A*).

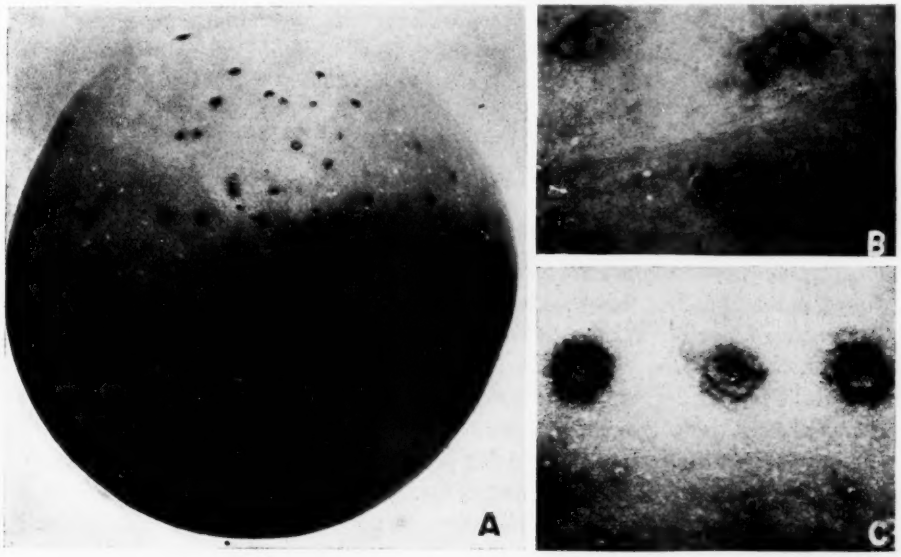

FIG. 1. BLISTER SPOT OF APPLES

A, Higginbotham apple showing natural infection. Spots roughly circular. Natural size.

B, Natural infection on Melon apple showing irregularly lobed appearance of blister spots. × 2.

C, White Pippin apple showing blister spots around inoculation punctures, three weeks after inoculation (August 27, 1916), with the blister-spot organism. × 4.

From a study of various stages on the same apple it was found that the first sign of the disease is a slight darkening around the lenticel, followed by the formation of a whitish to pale brown blister, 0.2 to 0.5 mm. in diameter. In later stages the epidermis over this blister becomes dark brown to black and dies. It may or may not crack loose from the healthy epidermis around it. If it does, the spot usually grows no larger; if it does not, the lesion may extend outward in various directions—Melon, Norfolk Beauty, Higginbotham, Klondyke—giving the irregularly lobed

appearance described above (fig. 1, *B*). On white or yellow apples such as Yellow Transparent the spots are surrounded by a narrow greenish ring; the same ring is found on Benoni and Duling, which are red when ripe, but the spots on Red Astrachan show a red ring.

The disease is not a serious one in the way that bitter rot is serious. Nevertheless, susceptible varieties often have the whole crop so seriously blemished as to be unfit for packing in any but the lower commercial grades. Fortunately such severe injury seems to be confined to varieties of minor commercial importance.

Cause of the disease

The disease is caused by an organism belonging to the genus Pseudomonas. It does not seem to have been described previously and the following name is proposed and a description appended:

Pseudomonas papulans n. sp.

Morphological characteristics. The organism is a rod, motile by one to six polar flagella. Flagella occur at both poles and were demonstrated by means of Loeffler's flagella stain, using stains on *Pseudomonas fluorescens* as check. (*Ps. fluorescens* furnished by the American Museum of Natural History, New York). Stained from twenty-four-hours-old agar cultures the organism shows as a short rod with rounded ends, measuring 0.9 to 2.3 μ long, by about 0.6 μ in diameter. It occurs singly and very often in pairs. No spores or capsules have been demonstrated. It stains readily with carbol fuchsin, gentian violet, and methylene blue. It is not acid fast and it does not stain by Gram.

Cultural characteristics. The following account is based on a study of 25 strains of the organism isolated from (1) naturally infected spots, (2) artificial infections, and (3) infections produced with the reisolated organism. Cultures were compared with *B. coli* and *B. amylovorus* (furnished by the American Museum of Natural History, New York).

The organism does not form gas from peptonized bouillon containing dextrose, saccharose, maltose, lactose, glycerin, or mannit, and it does not cloud the closed end of the fermentation tube in any case. Growth stops short in the neck of the tube, indicating an obligate aerobe. The organism clouds beef bouillon +10, slightly in twenty-four hours, and moderately in forty-eight hours. It liquefies gelatin slowly at 20°C., liquefaction not being complete in test-tube cultures until after twelve to fourteen weeks. Some strains form a soft coagulum in plain milk, some no coagulum at all, but all of them clear it in about twenty days. The organism blues litmus milk throughout during the first six days,

with the formation usually of a soft coagulum, and then gradually decolorizes it from above, with the production of a dark blue[3] color throughout after sixteen to twenty days. On plain agar it produces a filiform, slightly convex, whitish growth. On potato cylinders it produces, after forty-eight hours, a whitish, filiform, irregularly spreading growth, which after seven days shows a slight browning, accompanied by a slight darkening of the medium. The optimum temperature seems to lie between 25° to 28°C., though fairly good growth takes place at 20°C. It does not grow at 37°C. The thermal death-point has not been determined. On dextrose agar and glycerin agar the organism produces a light green fluorescence as also in Uschinsky's and asparagin solutions, but it does not grow in Cohn's solution. A test with six strains showed that the organism grows in bouillon over chloroform and tolerates hydrochloric acid up to +15 on Fuller's scale, and sodium hydroxide to −5. The optimum reaction for growth seems to be about +10.

The organism is sensitive to sunlight. Petri dishes one-half covered with black paper and exposed to sunlight, on August 3, on a bag of crushed ice showed, for six strains, an average of 98 per cent killed after an exposure of ten minutes. Six strains inoculated into test-tubes containing different amounts of sodium chloride showed growth in all up to and including 4 per cent. Using the method described by Edson and Carpenter[4] it was found that the organism produces alkali in plain milk during the first ten days, then increasing amounts of acid up to forty days. On peptonized bouillon containing two per cent of various sugars and alcohols it produces acid from dextrose and saccharose, alkali from lactose, and maltose, and neither acid nor alkali from glycerin or mannit.

Quick tests for differential purposes; bluing of litmus milk followed by decolorization from bottom upward accompanied by slow digestion and the formation usually of a soft coagulum; fluorescence and luxuriant rugose growth on glycerin agar; fluorescence on neutral gelatine but none on gelatine +10.

Isolation of the organism. The organism was easily isolated from affected apples by the method of poured agar plates. Spots were merely given a good washing with sterilized, distilled water, sometimes preceded by a brief rubbing with a finger dipped in alcohol. The diseased material was then scraped off with a sterilized scalpel and dropped directly into melted agar. The colonies appeared in from thirty-six to forty-eight hours, usually in pure culture. They were thin, smooth, circular, glistening,

[3] Saccardo, P. A. Chromotaxia seu Nomenclator Colorum. 1–22. 2 pl. Pata vii, 1894.

[4] Edson, H. A. and Carpenter, C. W. Micro-organisms of maple sap. Vermont Agr. Exp. Sta. Bul. **167**: 321–610. 1912.

whitish by reflected light, bluish by transmitted light, and 0.1 to 1.0 mm. in diameter. Not all the affected varieties were used in this work but no difficulty was experienced in obtaining pure cultures from those that were used.

Inoculation. Using sub-cultures from single colonies, the disease has been reproduced on six varieties of apples. In this work 123 apples were used, 60 of which were checks. All of them were treated on the trees and were bagged after treatment. The incubation period averaged about fourteen days, though some apples failed to show signs of the disease until the end of eighteen to twenty-five days. Some strains of the organism were infectious on all the varieties tested, others on only one or two. Further work is necessary to clear up this situation.

Inoculations were made in three different ways, using twenty-four to forty-eight-hours-old bouillon cultures:

1. By spraying uninjured apples with the bouillon culture. No infection resulted.

2. By spraying apples which had first been pricked with a flamed needle. Seventy-two per cent infection resulted on Yellow Transparent, Jonathan, Melon, Hawley, and White Pippin. No signs of infection appeared on Benoni.

3. By hypodermic injection just under the epidermis. Eighty per cent infection resulted on Benoni, Hawley, Jonathan, Melon, and White Pippin.

Inoculations with reisolated cultures by hypodermic injection on White Pippin, Jonathan, and Melon were also successful. The organism isolated from infections agreed in all characteristics, morphological and cultural, with the one used for inoculation. Checks to correspond with the three methods of inoculation described above remained healthy throughout the season.

ROUGH-BARK OR SCURFY-BARK CANKER OF APPLES

Description of the disease

What might be called the quiescent stage of the scurfy-bark disease occurs as patches of roughened scaly bark which somewhat resemble blotch canker (*Phyllosticta solitaria* E. and E.), but differ from it in showing no blotch pycnidia and usually no regularity of cracking up and down or across the limb (fig. 2, *A*). These roughened patches vary greatly in size from those covering only a few square centimeters to those covering the whole side of a limb for a meter or more. They are usually found on the north side of a limb and with few exceptions are bordered by a pimpled

lumpy ridge slightly more brown or reddish brown than the healthy bark around them.

The active stage of the disease, occurring in early spring, is characterized by a loosening and sloughing off of the outer bark, quite unlike anything that takes place in any other canker known to the writer. It reaches

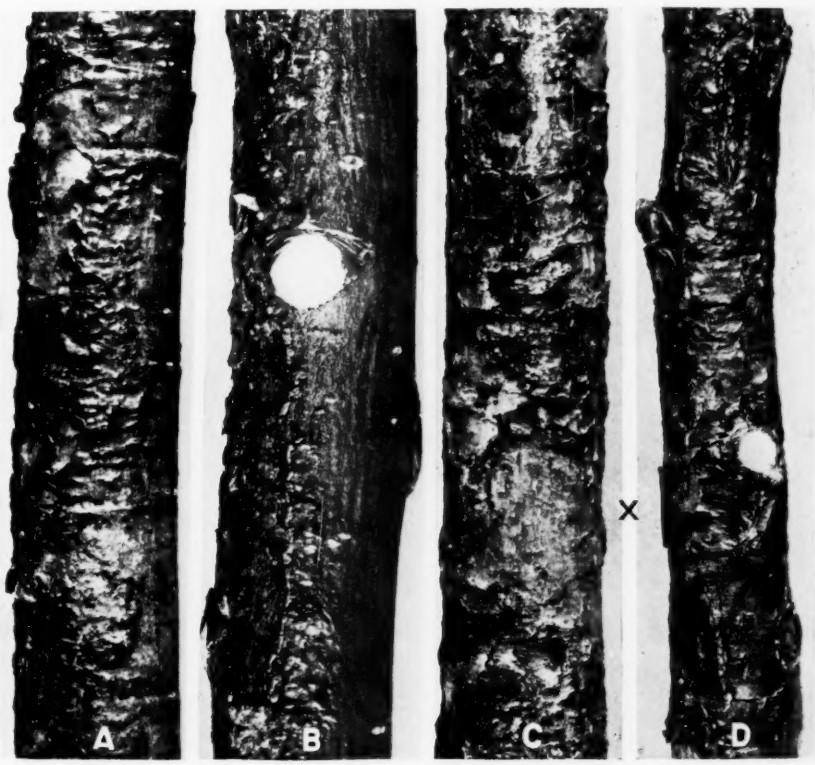

FIG. 2. SCURFY-BARK CANKER ON WHITE TRANSPARENT (DWARF) APPLE

A, *B*, Pimpled lumpy ridge at the edge of roughened areas. *C*, typical roughening, with an area exposed by deep-peeling, at *x*. *D*, the more common type of roughening, without marked peeling of affected bark.

its extreme form on certain varieties of dwarfs but has been observed on Logan, Munson, Ben Davis, and several others. For convenience of reference it wi l be known in this paper as the "deep-peeling" type of the disease.

In the development of this active stage, the first change visible from the outside is the formation of narrow cracks, 1.0 to 1.5 mm. deep by 5 to 20 cm. long, about 1 cm. outside of the pimply ridge referred to above

(fig. 3, *A*). Inside of these cracks the bark is found loosened—as early as March 21, 1916—in a layer about 2 mm. deep. It is easily peeled off and if this be done there is revealed a spongy layer of giant cells (fig. 3, *B*) about 1 mm. thick, which is white or greenish white at first, but which

FIG. 3. SCURFY-BARK CANKER

A, Limb of White Transparent (dwarf), natural size, showing typical loosening of bark by "deep-peeling" type of scurfy-bark canker. Photograph made April 25, 1916. *B*, Portion of another limb, with loosened layer removed, showing spongy layer beneath. Photograph made April 25, 1916. × 1.5.

C, Limb of Count of Wick (dwarf). Photograph made August 19, 1916, three months after inoculation with strain A 9 isolated from diseased bark. Natural size.

soon oxidizes to a pale brown and later to a dark brown color. Under natural conditions the loosened outer layer begins to dry and curl slightly within a few days after the cracks are formed; as a consequence the cracks widen, air circulates more freely under the loosened layer and the spongy layer dries down. Within two weeks from the time the cracks first show there is usually no spongy layer to be found and the loosened layer has become dried, curled fragments which break off almost at a touch. Many varieties show no wholesale loosening of the outer bark, but merely a

scaling off of small patches without the formation of a definite spongy layer. Sometimes the dry, brown vestiges of such a layer can be found, sometimes not. Possibly in such cases it develops slowly and progressively from one point to another, loosening the bark only a little at a time and drying down almost immediately.

No detailed studies of the diseased tissues have been made. A preliminary survey of both free-hand and microtome sections shows the presence of peculiar spots or pustules like those described by Hewitt[5] in his work with a new disease of apple bark in Arkansas and by the writer[6] in work with what is almost certainly the same disease, known at this station for over ten years under the name of pimple canker. It remains to be proved, however, whether this disease is a phase of or in any way related to the true scurfy bark canker. The most that can be said at present is that they show considerable morphological similarity and are often though not always associated on the same tree.

Besides the deep-peeling type of scurfy-bark canker there sometimes occurs a "shallow-peeling" type, in which only the epidermis is loosened. A spongy layer is formed here also, but it is thinner and more evanescent than in the deep-peeling type.

Occurrence of the disease

The disease occurs on such standard varieties as Ben Davis, Jonathan, Logan, White Winter Pearmain, Beach, Stayman Winesap, Munson, and Marsh, but much more severely on certain dwarf varieties.

The writer has found the disease in numerous orchards in southern Missouri and has received specimens of it from perhaps a dozen localities scattered over the state. No information is at hand as to is occurrence in other states, unless the trouble investigated by Hewitt in Arkansas be considered a phase of it. Affected trees are not quickly killed as in the case of Illinois canker—caused by *Nummularia discreta*—but there is no doubt that the peeling off of fresh layers of bark every spring is definitely injurious to the tree, aside from the opportunity given for entrance of canker fungi and various bark insects.

Cause of the disease

The disease seems to be caused by the same organism as that of the fruit spot. Poured agar plates, using material from (1) the deep lying

[5] Hewitt, J. Lee. An unknown apple disease. Arkansas Exp. Sta. Bul. **122**: 481–491. 1912.

[6] Rose, Dean H. Report of the Pathologist. Missouri State Fruit Exp. Sta. Rept. **1913–14** (Bul. 24): 30. 1914.

spongy layer, (2) the pimply ridge at the edge of roughened areas, (3) areas exposed naturally the preceding season when the loosened layer peeled off, (4) the spongy layer under loosened epidermis, gave practically pure cultures of an organism very similar, morphologically, to the blister-spot organism. Extensive cultural studies show, however, that instead of one organism there are two different ones or possibly two closely related but distinct varieties. One of them, represented by five strains, shows great similarity in morphological and cultural characteristics to the blister-spot organism; the other, represented by fifteen strains, resembles the blister-spot organism morphologically but differs from it in cultural characteristics. It liquefies gelatin rather rapidly, it produces a green fluorescence on nutrient agar and it begins to clear litmus milk inside of twenty-four to thirty-six hours. The first and second of these are not characteristic of the blister-spot organism, while the last is characteristic only of three strains.

Inoculations

Inoculation of bark with the rapidly liquefying bark organism produced swollen spots 1 to 2 mm. high and covering an area of roughly 1 sq. cm. (fig. 3, *C*). At these swellings the typical signs of the disease were reproduced, in miniature, and an organism was recovered which agreed in cultural and morphological characteristics with the one used for inoculation. The checks showed only a slight swelling. Typical blister spots on the fruit were produced by inoculating Jonathan and Melon apples with both bark organisms and these in turn were recovered from the lesions produced. While such evidence is strongly suggestive it is not final proof, and more work is necessary before the true relation between the fruit disease and the bark disease can be discovered.

In table 1 are shown the results of comparative tests made with the blister-spot organism and the rapidly liquefying bark organism.

A similar comparison between the bark and the blister-spot organism on the one hand and *Pseudomonas fluorescens* on the other show so many differences that the two former must be considered entirely distinct from the latter.

Further work on these diseases should include (1) a continuation of cultural studies of the blister-spot organism and the two bark organisms, (2) cross-inoculations from bark to fruit and from fruit to bark, (3) a study of the time and mode of infection and (4) a study of the microscopical characteristics of healthy and diseased tissues.

TABLE 1

Comparison of the blister-spot organism and the bark organism

	BLISTER-SPOT ORGANISM[1]	BARK ORGANISM[2]
Agar plates	Whitish colonies, slightly bluish in transmitted light; medium not greened	Whitish colonies, slightly bluish in transmitted light; medium slightly greened
Agar slant	Growth rather slow, no fluorescence	Growth rather slow, fluorescence marked
+ 10 bouillon	Clouding and pellicle; one strain showed faint green fluorescence in 2 weeks	Clouding; green fluorescence after 1 week; four strains showed pellicle
Gelatin stab	Liquefaction slow, begins in 2–3 days. Complete in 12–14 weeks. No fluorescence	Liquefaction more rapid, begins in 24 hours, complete in 2 months. Slight fluorescence after 1 week
Neutral gelatin stab	Liquefaction slow. Slight fluorescence	Liquefaction more rapid. Marked fluorescence
Litmus milk	Alkaline reaction for 10 days, then slow increase in acidity up to 40 days. Medium cleared in 2–3 weeks; final color blue throughout. Banded appearance produced by 3 strains	Slight alkaline reaction at top in 2 days, acid reaction below. Acidity increases as digestion proceeds. Banded appearance resulting in clearing in 3–4 weeks. Final color greenish blue above, buff below
Sterile milk	Clearing begins in from 7–10 days, complete in 17–20 days	Clearing begins in 36 hours, complete in 3–4 weeks. Green fluorescence
Glycerin agar	Growth vigorous, elevated, contoured to rugose. Moderate fluorescence	Growth vigorous, elevated, contoured to rugose. Fluorescence more marked than for blister-spot organism
Uschinsky's solution	Clouding moderate; bluish green fluorescence. Pellicle of pseudo-zoogleae-like fragments	Same as for blister-spot organism except fluorescence more marked with some strains
Nitrate reduction	None	None
Indol test	No indol in 20 days	Indol present in 10 days
Ammonia test	Ammonia produced	More ammonia produced than by the blister-spot organism
Resistance to sunlight	Average of 98 per cent killed after 10 minutes exposure	Average of 50 per cent killed after 10 minutes exposure
Flagella	Bipolar; one to several	Bipolar; one to several

[1] Under this heading are included also the slow liquefiers from bark.

[2] Under this heading are included only the rapid liquefiers from bark.

METHODS AND MEDIA

As standards for methods and for the making of media the writer has followed directions given in Erwin F. Smith's Bacteria in Relation to Plant Diseases, Vol. I, and Eyre's Bacteriological Technic, 2nd Ed., except where noted otherwise in the text.

SUMMARY

1. In the foregoing paper is described a bacterial disease of apples, no mention of which has been found in phytopathological literature.

2. By isolation, cultural, and inoculation work, it is proved that this disease is caused by a motile organism, which liquefies gelatin slowly, and belongs to the green-fluorescent group of bacteria.

3. Because of the blister spots produced by this organism on the surface of apples, the name *Pseudomonas papulans* is proposed.

4. Description is also given of a rough-bark or scurfy bark canker from which has been isolated an organism also belonging to the green-fluorescent group.

5. Evidence is presented that there are really two varieties of the bark organism, one of which has all of the cultural characteristics of the blister-spot organism, including slow liquefaction of gelatin, while the other differs from it in several important particulars, including rather rapid liquefaction of gelatin.

6. Inoculation of healthy apple bark with two strains of the rapidly liquefying bark organism produced small lesions which showed the typical cracking loose of diseases from healthy bark, and in several cases the lumpy appearance characteristic of the early stages of the scurfy-bark canker.

7. An organism agreeing in cultural reactions with the one used for inoculation has been recovered from these lesions.

8. Typical blister spots have been produced by inoculation of both types of bark organisms into healthy apples. From these spots the organisms used for inoculation have been recovered.

9. A preliminary comparative study of the cultural characteristics of the blister-spot organism and the two bark organisms suggests that the difference between them are differences of degree rather than of kind. That is, that all three are possibly merely varieties of one species. More work is necessary, however, before this question of relationship can be settled.

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THE PATHOGENIC ACTION OF RHIZOCTONIA ON POTATO

H. T. Güssow

With One Figure in the Text

It appears that satisfactory evidence has been lacking clearly demonstrating the pathogenic action of Rhizoctonia (*Corticium vagum* B. & C.) on various host plants, particularly the potato.

The attention which the well known disease has received in the new world and more recently in the old, left, in my opinion and in that of quite a number of other investigators, several important points unexplained. Every pathologist fully recognized the symptoms of this disease which have come to be regarded as typical Rhizoctonia infection. The unmistakable folding of the leaves of the growing plant, together with the brown stem lesions so frequently described, in other instances, or in addition perhaps, the formation of aerial tubers and peculiar smallness of subterranean tubers, are now well known as general symptoms resulting from an attack of Rhizoctonia.

Indeed, the folding of the leaves associated with this disease differs greatly from the curling of the leaves of plants affected with leaf roll, particularly noticeable is this difference when examining the lower leaves of an affected plant. Where, however, doubt existed, the presence of stem lesions was looked upon as final proof of a Rhizoctonia infection.

I must confess, however, that the often surprising scarcity and apparent superficiality of these lesions, nay, often enough their entire absence in what was otherwise unmistakably a plant infected with Rhizoctonia and not with leaf roll, frequently caused me surprise and certainly failed to readily convince the farmers on the occasion of field demonstrations of the correctness of the diagnosis—which at times I shared much against my own belief.

For some time I have endeavored to discover the true pathogenic action of this fungus on the host plant. My colleague, Drayton,[1] demonstrated to my satisfaction the profuse permeation of the tissues of and surrounding the lesions, but even that failed to convince me entirely of the cause of the characteristic symptoms, which statement is not intended to infer

[1] Drayton, F. L. The Rhizoctonia lesions on potato stems. Phytopath. **5**: 59. 1915.

that I doubted Rhizoctonia to be associated with the same. But from what evidence was available, the actual injuries caused by the stem lesions were so infinitesimal that it was felt the true injury is done elsewhere, and what we did observe was the result of such unlocated but far more serious injury.

A careful study of diseased plants in the field revealed at first little or no additional clues. Lesions were sometimes present where the leaves were folded, the tubers were covered with more or less numerous lumpy fungous masses, indeed the roots often showed the well known pseudosclerotia. Pot experiments showed the presence of sclerotia on rootlets more abundantly than was the case in the field, and yet while abundant superficial and lesser amounts of intracellular hyphae of Rhizoctonia were found on microscopical examination, the evidence of an all round general soundness of the underground parts examined still left the seat of the injury undetermined.

On careful examination of the root system of a plant clearly affected with Rhizoctonia and no other disease, that had been pulled up from loose sandy soil, or had been lifted with care by means of a fork or spade, one factor at last attracted my attention, which later led to interesting observations. This was the almost entire absence of the fine fibrous rootlets, so common in sound plants. Surely such rootlets must have been present originally? When examining thereafter plants in various stages of infection one could observe a corresponding absence or presence of finer rootlets according to the amount of disease present. Of course, in this determination care is necessary, but after some experience one cannot but recognize the existing relation of rootlets to degree of disease.

What if the fungus acted upon the roots of the growing plant similar to the way in which it does on the roots of Rhizoctonia-infected tubers sprouted in a closed stender dish? The disastrous effects of the fungus at the early stages of growth are sometimes so pronounced as to kill off growth altogether; this is a well known fact.

Let us bear in mind that in a potato field we find many stages of severity of Rhizoctonia infection, from total "misses" to one, two or more shoots clearly affected up to the case where the plant bears plenty of aerial tubers and numerous little potatoes underground from which the popular name "little potato disease" has sprung. Aerial tubers have been commonly associated with Rhizoctonia, they are perhaps exclusively manufactured from material produced by the leaves, a comparatively slow process, but always indicating impaired root function in plants where they may be considered abnormal. Aerial tubers naturally may occur from any cause cutting off or interrupting root function, but only when such interruptions are gradual. We have, therefore, no aerial tubers in the black

FIG. 1. RHIZOCTONIA LESIONS ON ROOTS OF POTATO

A, Root of potato fourteen inches in length showing at *s*, sclerotial deposits and at *x*, many places where the fine absorbing roots were killed by the action of the fungus. Slightly reduced.

B, Portion of root shown in *A*. × about 1.5.

leg disease, where the cutting off of supplies is rather sudden. All these symptoms are the logical results of the absence of the abundant feeding roots. Roots are present in all growing plants, otherwise the plants would have died; small and fine roots are less in evidence in affected plants while a generous supply exists in healthy strong plants.

Deductions—however logical they may be—still are hypotheses and hypotheses are not facts, but the accompanying plate will provide some foundation for the observations recorded and may stimulate wider researches on this point than have been made so far. I am satisfied from the observations made, that the destruction—often very gradual—but very persistent all the same, of all or many of the feeding roots of the potato plant accounts for every one of the symptoms associated with this disease. The lesions which have so often been recorded are evidently not of serious consequence, as indicated by their general superficiality and frequent entire absence. In some instances indeed these lesions are not due to Rhizoctonia at all, but to *Actinomyces scabies* Güssow, which I hope to show in another paper, when they afford easy resting places in the unprotected superficial cells for the mycelial masses of Rhizoctonia shown in Mr. Drayton's photo-micrographs, as well as for the permeation of the hyphae into the interior, which, as must have been noticed, is not accompanied by any prominent injurious action upon the cells invaded. A study of Mr. Drayton's slides clearly confirms this observation as well as the photographs made from them which are accessible to our readers.

The pathogenic action is as follows: We are aware of the very profuse growth of mycelium of Rhizoctonia, particularly in the dark, as also of the production of enormous quantities of pseudo-sclerotia on roots and tubers. Whether the sclerotia are left over in the soil from preceding potato crops or other host plants, or whether they have been introduced by untreated infected seed potatoes—(and what "farmers' run" potatoes are not infected?)—does not matter much. The tips of the fresh rootlets soon fall a victim to the invading mycelium, the root cap being undoubtedly the most vulnerable point and soon the short roots have been destroyed, the mycelium meanwhile reaches older rootlets, which it much more rarely destroys, though that has occurred, but where the mycelium frequently produces resting mycelial masses from which invading hyphae issue almost simultaneously with new rootlets which are produced by the plant in its effort to reestablish its resources. This process goes on gradually and slowly or more rapidly depending naturally upon the vigor of the plant. Finally the persistent efforts of the fungus result in decreasing yields, in frustrating the growth of the tubers, because of lack of food supplies from the roots, and eventually in the production of aerial tubers. Meanwhile harvest-time has arrived, what tubers are there, are harvested,

but the roots infected with sclerotia remain in the ground, since they are not pulled up by the digger or are at any rate returned to the ground. With the diminishing food supply in these roots, sclerotia develop ready for subsequent attacks. This observation also accounts largely for the soil contamination and the persistence of the organism in land once infected. It also indirectly suggests a new means of control, viz., the prevention of infection by cultural methods or the application of fertilizers producing vigorous plants in the first instance and aiding in the production of a generous supply of new feeding roots.

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OTTAWA, CANADA

SYNTHETIC CULTURE MEDIA FOR WOOD-DESTROYING FUNGI[1]

ERNEST J. PIEPER, C. J. HUMPHREY AND S. F. ACREE

Wood-destroying fungi grow readily on many of the ordinary culture media which have as their principal ingredients malt extract or plant decoctions and meat extracts, usually hardened with agar-agar or gelatin. As an example of such media, a malt extract preparation of the following formula has proved very satisfactory for general culture work:

Extract of 1 pound lean beef[2] in distilled water	1000 cc.
Malt extract	25 grams
Agar-agar	20 grams

This has found a wide use in Europe and has been employed for much of the routine work in the pathological section of the Forest Products Laboratory. In certain lines of investigation, however, such as the testing of the toxicity of chemical substances, and in comparative tests on the physiological behavior of wood-destroying fungi, this medium has the serious disadvantages of being chemically complex and variable. Its composition and constitution depend upon the nature of the meat and malt extract, the method of preparation and the duration of standing, and physically it may be variably colloidal. The possibility of chemical or physical combination of certain preservatives with the highly complex organic compounds and also the coagulation of the latter by electrolytes are of extreme importance also in toxicity work.

In the present work an attempt was made to prepare a synthetic medium which would support a growth of wood-destroying fungi at least as good as that on the malt extract agar, and which at the same time would be composed of as simple constituents as possible. The medium could then be duplicated at any time, by any investigator, provided the chemicals used were of the same standard of purity.

For satisfactory growth of wood-destroying fungi a culture medium must have, in addition to certain simple inorganic salts, the necessary compounds to furnish both nitrogen and carbon in a form readily available to the fungus. Ammonium salts, nitrates and asparagin or its salts have

[1] The present paper is one of four prepared by the junior author (Pieper) in partial fulfillment of requirements for the degree of Doctor of Philosophy in the University of Wisconsin.

[2] Later tests in this laboratory indicate that beef is of little or no advantage; in many cases it somewhat retards the growth of wood-destroying fungi.

frequently been used in synthetic media as a source of nitrogen, while various carbohydrates have been used as a source of carbon.

The first step in this work was to select a nutrient solution of inorganic salts with di-ammonium phosphate as the source of nitrogen. This solution was used as the basis for testing the nutrient value of various carbohydrates and consisted of the following:

Di-potassium phosphate (K_2HPO_4)	4 grams
Di-ammonium phosphate ($(NH_4)_2HPO_4$)	2 grams
Magnesium sulphate ($MgSO_4.7H_2O$)	2 grams
Agar-agar (powdered)	15 grams
Distilled water	1000 cc.

Varying concentrations of the following carbohydrates, usually 20 or 40 grams in a liter of the nutrient solution, were used: Lactose, maltose, cane-sugar, galactose, glucose and glucosamin. Growth of the fungus[3] was compared with that on malt extract agar as a standard.

The organism grew fairly well in every case. Cane-sugar and glucose, however, gave the most favorable indications, the growth on the cane-sugar being slightly more favorable. Since, however, the growth might be changed by using other nitrogen sources in addition to di-ammonium phosphate, it was decided to continue the use of both sugars in further work.

The next step was to obtain a nitrogen source more available to the fungus than di-ammonium phosphate, so the following substances were tested by adding 2 and 4 grams, respectively, to a liter of the nutrient solution containing forty grams of cane-sugar: Asparagin, sodium asparaginate, ammonium asparaginate, caffein, guanidin carbonate, glycin, leucin, creatinin and betain. With 0.2 and 0.4 per cent of caffein and guanidin carbonate, respectively, no growth of the fungus occurred, but in all the other cases a fairly good development was secured. The best growth was, without doubt, obtained with asparagin and its sodium and ammonium salts. The fact that asparagin alone might be a source of available carbon for fungi was considered, but experiments showed that practically negative results were obtained in the absence of a sugar.

Glucose was next substituted for cane-sugar while using asparagin and di-ammonium phosphate as a source of nitrogen. The character and rate of growth was the same as that obtained with cane-sugar. It has been shown[4] that not all fungi contain an enzyme which will hydrolyze cane-sugar, hence the substitution of glucose would presumably be an advantage, and cane-sugar was therefore discarded.

[3] *Fomes annosus* was used in all the preliminary tests.

[4] Boeseken, J., and Waterman, H. Akad. Wetensch. Amsterdam, **20**: 548. 1911. Abstract in Bot. Gaz. **59**: 413. 1915.

On the basis of these experiments a culture medium of the following composition was selected for further test:

Glucose ($C_6H_{12}O_6$), powdered	40.00 grams
Di-potassium phosphate (K_2HPO_4)	4.00 grams
Asparagin ($C_4H_8O_3N_2$)	4.00 grams
Di-ammonium phosphate ($(NH_4)_2HPO_4$)	2.00 grams
Magnesium sulphate ($MgSO_4.7H_2O$)	2.00 grams
Calcium carbonate ($CaCO_3$)	0.25 gram
Calcium chloride ($CaCl_2$)	0.10 gram
Agar-agar (powdered)	15.00 grams
Distilled water	1000.00 cc.

It is evident that this medium is synthetic except for the agar-agar[5] which is used as a solidifying agent. Its nutritive properties for four important wood-destroying fungi were determined. As a comparison, similar tests were made on another medium differing only in the substitution of Witte's peptone for asparagin and di-ammonium phosphate. Although this latter medium is not strictly synthetic, still a definite grade and purity of the peptone can be obtained from a reliable source. For many purposes such a medium might be found of considerable advantage, as has been shown by tests on *Fomes annosus* and *Fomes pinicola* reported later.

In preparing the synthetic medium, the mixture was heated in a 1.3-liter flask in a water bath to prevent charring. It was then poured into test-tubes as quickly as possible, and well stirred during the operation to avoid losing any precipitate that was formed. The tubes were then plugged with cotton and sterilized in live steam (100°C.) without pressure for thirty minutes on three successive days. A very slight precipitation was found in the bottom of the tubes after sterilization. This was probably a mixture of calcium and magnesium phosphates and carbonates. As small amounts of calcium salts seem to increase the vigor of the growth, it is advisable to retain this sediment by thoroughly shaking the tubes before pouring into petri dishes.

The Witte's peptone medium was prepared in the same manner as the synthetic and was composed of the following substances:

Glucose ($C_6H_{12}O_6$), powdered	40.00 grams
Di-potassium phosphate (K_2HPO_4)	4.00 grams
Witte's peptone	4.00 grams
Magnesium sulphate ($MgSO_4.7H_2O$)	2.00 grams
Calcium carbonate ($CaCO_3$)	0.25 gram
Calcium chloride ($CaCl_2$)	0.10 gram
Agar-agar (powdered)	15.00 grams
Distilled water	1000.00 cc.

[5] Colloidal silicic acid has been used by some workers in place of agar-agar and might be of advantage here but the writers have not experimented with it.

The malt extract medium used for comparison was made as previously indicated. It was tubed and sterilized in the same manner as the others.

All these media were tested with four wood-destroying fungi. The tables give the growth of these at 25°C. over varying periods up to twenty-eight days.

From the results it is seen that the synthetic culture medium which produced the best results gives a good growth and compares favorably with the malt-extract medium. Perhaps by continued cultivation the fungi may gradually become adapted to the new medium, giving then better results than were obtained in this investigation.

The Witte's peptone medium is especially good for the growth of *Fomes pinicola* and *Fomes annosus*. With *Lenzites sepiaria*, and especially with *Stereum frustulosum*, it gave less favorable results than the other two.

By additional work it may be possible to improve the synthetic medium further so that it will give a better and more uniform growth than here described. Such a medium would be very valuable both for toxicity work and general physiological experimentation with wood-destroying fungi.

In addition to the media for which the formulae are given in the present paper sixteen other preparations were tried. The proportions given are for 1000 cc. distilled water and 1.5 per cent agar. None of these were as satisfactory as those reported above.

1. Cane sugar, 40 grams; ammonium asparaginate, 4 grams; magnesium sulfate, 2 grams.

Gave thick but slow growth for *Fomes pinicola;* with *Fomes annosus* gave thin growth, covering the plate in two weeks.

2. Fraenkel and Voges' Solution.[6]

Gave very poor growth.

3. Fermi's Culture-Fluid.[6]

Gave poor thin growth; plate not covered in three weeks.

4. Uschinsky's Solution.[6]

Gave poor thin growth; plate not covered in three weeks.

5. Modified Uschinsky's Solution.[6]

Gave very poor growth.

6. Hasselbring's Solution.[7]

7. Cane sugar, 40 grams; glycerin, 40 grams; asparagin, 4 grams; magnesium sulfate, 2 grams; di-potassium phosphate, 4 grams; di-ammonium phosphate, 4 grams.

Gave best growth for *Fomes annosus;* thin growth for *Fomes pinicola;* thick but slow growth for *Fomes applanatus, Lenzites sepiaria* and *Stereum frustulosum.*

[6] See E. F. Smith, Bacteria in relation to plant diseases, Vol. I, p. 197, 1905.

[7] Glucose, 1 gram; ammonium nitrate, 1 gram; di-potassium phosphate, 0.5 gram; magnesium sulfate, 0.25 gram.

TABLE 1

Radial growth in millimeters of Fomes annosus in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS		
		7 days	10 days	14 days
Synthetic	1	21–23	30–34	40–42*
	1′	23–24	31–35	40–48*
Peptone	2	23–24	35–37	42–44*
	2′	24–26	33–39	40–42*
Malt-extract	3	24–25	29–34	40–43*
	3′	26–28	30–35	41–45*

Character of growth:

Synthetic. Slightly more fluffy and slightly less dense than on malt-extract, although the color was the same.

Peptone. About the same as on synthetic medium but not quite as dense.

Malt-extract. Dense, creamy, fluffy growth.

The growth of *Fomes annosus* on synthetic medium and peptone medium was tested for acidity with litmus and found to give a slight acid reaction after fourteen days growth.

*Surface of medium entirely covered.

TABLE 2

Radial growth in millimeters of Fomes pinicola in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS			
		7 days	10 days	14 days	21 days
Synthetic	1	24–28	29–32	33–35	Surface covered
	1′	24–26	27–33	30–35	Surface covered
Peptone	2	21–22	31–32	Surface covered	
	2′	21–22	31–32	Surface covered	
Malt-extract	3	26–27	32–33	Surface covered	
	3′	25–27	32–34	Surface covered	

Character of growth:

Synthetic. Thin and striated, color same as on malt-extract medium.

Peptone. Very fluffy and just as dense as on malt-extract medium.

Malt-extract. White, dense, fluffy growth.

TABLE 3

Radial growth in millimeters of Lenzites sepiaria in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS				
		7 days	10 days	14 days	21 days	28 days
Synthetic	1	21–22	30–31	33–37	Surface covered	
	1′	22–23	31–32	37–38	Surface covered	
Peptone	2	17–20	28–30	34–35	37–39	Surface covered
	2′	16–20	29–31	35–36	36–39	Surface covered
Malt-extract	3	17–18	27–30	33–35	Surface covered	
	3′	17–20	29–30	36–38	Surface covered	

Character of growth:

Synthetic. Margin of growth thin with much sub-growth; more fluffy towards center than on malt-extract; color not as dark as on malt-extract medium.

Peptone. Growth poor in appearance; color same as on malt-extract medium.

Malt-extract. Fairly dense; dirty brown color.

TABLE 4

Radial growth in millimeters of Stereum frustulosum in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS				
		7 days	10 days	14 days	21 days	28 days
Synthetic	1	9–12	14–16	29–33	Surface covered	
	1′	10–11	15–19	28–34	Surface covered	
Peptone	2	4–5	5–6	8–9	9–10	10–11
	2′	7–9	9–11	10–13	12–13	13–14
Malt-extract	3	11–16	21–22	26–28	30–36	Surface covered
	3′	13–14	22–23	27–29	36–37	Surface covered

Character of growth:

Synthetic. Very fluffy, dense and creamy; slightly more brown in color than on malt-extract medium.

Peptone. Poor growth; deep orange-brown color.

Malt-extract. Fluffy growth; slight yellow tint.

8. Differs from No. 7 in substitution of 40 grams galactose for the cane sugar.

Gave much slower growth than No. 7; plate not quite covered in three weeks.

9. Differs from No. 7 in substitution of 40 grams lactose for the cane sugar.

Gave slow growth, not as good as No. 8; plate not covered in three weeks.

10. Cane sugar, 40 grams; glycin, 4 grams.; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Gave good growth; almost as rapid as on malt-extract agar, but appearance not as good as on No. 1.

11. Differs from No. 10 in substitution of 4 grams creatinin for the glycin.

Gave fair growth; plate covered in three weeks.

12. Differs from No. 10 in the substitution of 4 grams. caffein for the glycin.

No growth in three weeks.

13. Differs from No. 10 in the substitution of 4 grams guanidin carbonate for the glycin.

Gave no growth in three weeks.

14. Cane sugar, 20 grams; glucosamin, 4 grams; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Fairly good growth, but less dense than on malt-extract agar; plate covered in two weeks.

15. Glucose, 25 grams; d and l-leucin, 4 grams; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Plate covered in two weeks but growth thinner than on No. 10.

16. Lactose, 32 grams; betain, 4 grams; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Growth fair; plate not quite covered in three weeks.

LITERATURE

As practically all of the literature dealing with synthetic culture media, as far as the writers have investigated, has reference to its adaptability to the growth of bacteria or molds and as these organisms apparently react to the media in a different manner than the wood-destroying Hymenomycetes upon which this study was conducted no bibliography is appended.

INVESTIGATIONS IN FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY
IN COOPERATION WITH THE FOREST PRODUCTS LABORATORY,
MADISON, WISCONSIN

PHYTOPATHOLOGICAL NOTES

Apple scab on the twigs. Does the apple scab (*Venturia pomi* (Fr.) Wint.) pass the winter on the twigs, which thus become a source of infection? This is a question frequently presented to the American plant pathologists. The presence of the organism in the twigs has been reported by a number of workers, but the first satisfactory data bearing on the probabilities of this being a source of infection were presented by Morse and Darrow in 1913.[1] They gave a very satisfactory review of the literature of the subject which will therefore be omitted in this discussion.

In the latter part of April, 1916, the attention of the writers was called to a number of diseased apple twigs from Freehold, Monmouth County, New Jersey. A careful examination showed that the organism was *Venturia pomi*, and that the conidia which were present in great numbers were viable.

The twigs showed an abundance of infection for several inches and the bark was split, thus giving them a very ragged appearance. Directly beneath the ruptured bark were dense masses of stromatic growth, which protruded through the openings and produced an abundance of conidiospores, which were short and irregular and rested on still shorter, in fact almost equilateral, irregular basal cells. The spores were typical and agreed in shape and size with those obtained from other sources, and grew readily in culture. We are unable to say whether these spores were formed in the fall of 1915 or the spring of 1916, but it is very evident that the organism survived the winter in the twigs.

The writers did not have an opportunity to examine the trees, but Mr. W. B. Duryee, Jr., the County Farm Demonstrator who sent the twigs to the Station, reported the disease in abundance. The twigs submitted to us carried an abundance of the organism, and the infection extended from the tip back some 12 to 15 inches, but was most severe near the tip.

Many of our New Jersey apple growers cultivate their orchards so

[1] Morse, W. J. Spraying experiments and studies on certain apple diseases in 1913. Maine Agr. Exp. Sta. Bul. 223. 1914.

Morse, W. J. Spraying experiments and apple diseases. Maine Agr. Exp. Sta. Bul. 252. 1916.

Morse, W. J. and Darrow, W. H. Is apple scab on young shoots a source of spring infection? Phytopath. **3**: 266–269. 1913.

thoroughly that fallen leaves are very scarce in the spring of the year. However, these same orchards will some times produce an abundance of diseased fruit. Although we fully realize that a severe infection may result from a small source, we have for some time been inclined to believe that there must be some source of infection other than the ascospores formed on the leaves of the preceding year.

MEL. T. COOK AND C. A. SCHWARZE

On using an ether freezing microtome in warm and damp weather. Most persons who use an ether freezing microtome may remember that during the warm and humid days of midsummer and early fall there is likely to be considerable difficulty in freezing material, when at other times little or no difficulty is experienced. During the very damp and rainy days of early summer (1916) much difficulty of this sort was experienced in the Laboratory of Forest Pathology, Providence, R. I. At times it was absolutely impossible to freeze the preparation. This led to some experimenting on the part of Mr. N. O. Howard, Collaborator, and the writer, which finally resulted in overcoming the main difficulty in a very simple and efficient manner. The apparatus consisted merely of a wide-mouthed, eight- or ten-ounce bottle containing anhydrous calcium chloride, which was inserted between the pressure tank and the atomizer. This bottle was connected so that the air from the tank passed through a glass tube in the stopper to the bottom of the bottle, and thence up through the mass of calcium chloride to another glass tube which connected with the atomizer by means of a rubber tube.

The calcium chloride was broken into small pieces and packed into the bottle, but not so tightly as to prevent the long glass inlet tube being worked down through the mass as the stopper was inserted. The rubber stopper was tied securely in place so as to prevent its being blown out by an excess of air pressure.

After a few days of occasional use the calcium chloride usually showed signs of deliquescence through absorption of moisture from the air. When this deliquescence became rather pronounced, the calcium chloride was placed in a small "fry-pan" and heated until it was again entirely dry and hard. After it had cooled sufficiently to handle it was broken up into small pieces and put back in the bottle while still warm. The same calcium chloride, which cost but a few cents when purchased, has now been in use for more than six months. When the freezing microtome was used several times each day the calcium chloride had to be dried about every week or ten days.

Although the apparatus described may be considered as a somewhat crude affair, it has worked very efficiently for more than six months and

has given no indication that its efficiency would not continue indefinitely. Without doubt a more finished apparatus of still greater efficiency would result from using a regulation chemical dehydrating apparatus.

Although this method of manipulation usually prevents the formation of snow on the under side of the freezing disk—which delays freezing—it does not always prevent it. Recent experiments by Mr. Howard show that this difficulty can be prevented entirely by placing several small lumps of anhydrous calcium chloride in the ether bottle, or, better, in the ether can itself as soon as it is opened, and letting it stand for half a day or a day before using. This withdraws the small amount of water in the ether, which apparently is partly responsible for the formation of the snow.

In using ether for freezing sections we have always found it necessary to filter the ether before it reaches the atomizer and also to avoid using rubber in contact with it. The ether intake tube in our microtome has an inside diameter of less than 2 mm., and filtering is very easily accomplished by thrusting a small wad of cotton into the end of the tube. This wad of cotton also is extremely useful in regulating the supply of ether going to the atomizer, as, with a little experience, the supply can be increased or decreased almost at will by using respectively a loose wad of cotton or a compact one. Of course the cotton used for filtering should be renewed whenever it shows any tendency to become clogged.

This note is offered for publication with the thought that other workers who have had similar difficulties in freezing material with ether might like to know that such difficulties can be overcome so easily.

J. FRANKLIN COLLINS

Note on Xylaria polymorpha and X. digitata. The recent article by Fromme and Thomas[1] on a root-rot disease of the apple in Virginia, in which the causal organism is provisionally referred to some species of Xylaria, may be further substantiated by the following observations.

In 1906, near Scottsburg, Indiana, the writer collected mature specimens of *Xylaria polymorpha* from diseased areas in living roots of a four-years-old apple tree of the variety Winesap. The following year this tree died and was pulled up. The conidial stage of the fungus was afterwards noted on the diseased roots. On October 5, 1908, in the same orchard *Xylaria digitata* was collected from the roots of a six-years-old pear tree which had died from some unknown cause. In the writer's herbarium are two other collections of *Xylaria digitata*, made at Priest River, Idaho, from decayed areas in living roots of *Populus trichocarpa* and *Cratægus*

[1] Science n. s. **45**: 93. 1917.

douglasii. The roots of the former were partially decayed, but not in the same part, by *Fomes applanatus*, and those of the latter by a species of Fomes[1] peculiar to this tree.

JAMES R. WEIR

Puccinia triticina Erikss. Leaf-rust of winter wheat causes damage in Kansas. It is generally considered that the leaf-rust of wheat due to *Puccinia triticina* Erikss. is not serious enough to cause any appreciable damage to the crop, at least publications indicate that an attack of leaf-rust in May or June does not produce any marked effect on the yield.

Observations by the writer the past season showed that the leaf-rust in some fields in Kansas was very abundant, and that its occurrence was not confined to the foliage but that the "necks" of the wheat were vigorously attacked by this rust. Careful observations and examinations of such fields showed that no other factors could have been responsible for the poor quality of the grain and the reduced yield. The yield of one variety in particular, a pure line winter wheat grown in Kansas and called P 706, was reduced 38 per cent, according to yield data furnished by the Department of Agronomy. The fields showing the effect most were those which had been planted late. It is thought that this is partially responsible for the large percentage of leaf-rust.

The percentage of infection on the "necks" of the wheat, as estimated by the newly adopted scale for estimating rust percentages of the Office of Cereal Investigations, Department of Agriculture, was 10 to 25 per cent, while the foliage of the above-mentioned variety generally showed 100 per cent of infection.

It is believed by the writer that the leaf-rust of winter wheat in Kansas can under favorable conditions cause considerable damage and that too little stress has been given in literature to the importance of this rust.

L. E. MELCHERS

Early discovery of white pine blister rust in the United States. There has recently come to the attention of the writer the fact that a specimen of white pine blister rust was collected on white pine (*Pinus strobus* Linn.) by Mr. Samuel N. Baxter of Philadelphia, in April, 1905, at a nursery near Philadelphia. A search of the correspondence in the files of the United States Department of Agriculture corroborates this statement.

The specimen which was sent to the United States Department of Agriculture was referred to the Mycologist, Mrs. F. W. Patterson, for

[1] Weir, James R. Notes on wood-destroying fungi which grow on both coniferous and deciduous trees. I. Phytopath. **4**: 272. 1914.

examination, and pronounced "a Peridermium which causes what is called a pine-blister rust."

A search in February, 1917, in the pathological collections of the Bureau of Plant Industry failed to reveal the specimen. The letter from Dr. L. O. Howard, dated April 22, 1905, referring the specimen to Dr. A. F. Woods, has the notation on it, "White Pine, Peridermium on" in the handwriting of the Mycologist.

Since there is no reason to believe that the determination was incorrect, and since but a single species of Peridermium has ever been reported as causing a blister rust on white pine, this specimen was in all probablity *Peridermium strobi* Klebahn, the pine stage of *Cronartium ribicola* Fisher. This record, then, antedates the finding by Stewart of the Cronartium stage on currants at Geneva, New York, in 1906, and the hitherto earliest record in this country of the Peridermium stage on pine discovered in New York on June 8, 1909, and reported by Spaulding in 1909.

ROY G. PIERCE

Needle rust on Pinus resinosa. In June, 1916, the writer saw at Sharon Vermont, a very striking case of needle rust in a plantation of 10,000 trees of *Pinus resinosa* about 4½ feet in height. Up to about 3 feet the yellow spore bodies, although small, were so abundant on the 1915 needles as to be readily discernible 15 to 20 feet away, single needles bearing as many as 20 pustules. Hedgcock has identified the rust as belonging to the two species, *Coleosporium solidaginis* (Schw.) Thum. and *C. delicatulum* (Long) Hedgc. & Long. The alternate hosts were abundant in the plantation as well as beyond it. This seems to be a case of healthy trees being brought to that locality and infected by the fungi which were already present on the herbaceous hosts. Because of the abundance of the rust the writer thought it might be a serious matter to such small trees. A second visit made in September, however, showed that the needles were a healthy green color except for small dead spots where the rust pustules were produced. It is possible that the damage may later become more evident and this point will be determined.

PERLEY SPAULDING

Notes on the distribution of the bacterial disease of western wheat-grass.[1] Until recently the writer had not observed the bacterial disease of western

[1] O'Gara, P. J. A paper read before the meeting of the American Phytopathological Society, Columbus, Ohio, December 28, 1915.
Abstract published in Phytopathology **6**: 98–99. 1916.
Science n. s. **42**: 616–617. 1915.
Phytopath. **6**: 341–349. 1916.

wheat-grass, *Agropyron smithii* Rydb., caused by *Aplanobacter Agropyri* O'Gara, outside of two counties within the state of Utah. The disease has been recently found, however, occurring on western wheat-grass in three widely separated districts of Montana, namely, Lewis and Clark, Broadwater, and Deer Lodge counties.

As noted in previous papers, this disease is most characteristic in that the yellow bacterial ooze is found to cover the glumes of the inflorescence and appears also in droplets of considerable size on the outside of the glumes and on the sheaths. It also causes some dwarfing of the plants as well as a bending of the stem above the last internode. When the inflorescence is infected germinable seeds are not produced.

The fact that this disease has been found in widely separated districts would indicate that it may be found wherever western wheat-grass grows. The writer would appreciate a note from anyone finding this disease, as he is interested in establishing the extent of is distribution.

P. J. O'GARA

The occurrence of Colletotrichum solanicolum O'Gara on eggplant. A note concerning this species of Colletotrichum was published as an abstract in Phytopathology.[1] Later a description of the organism appeared in

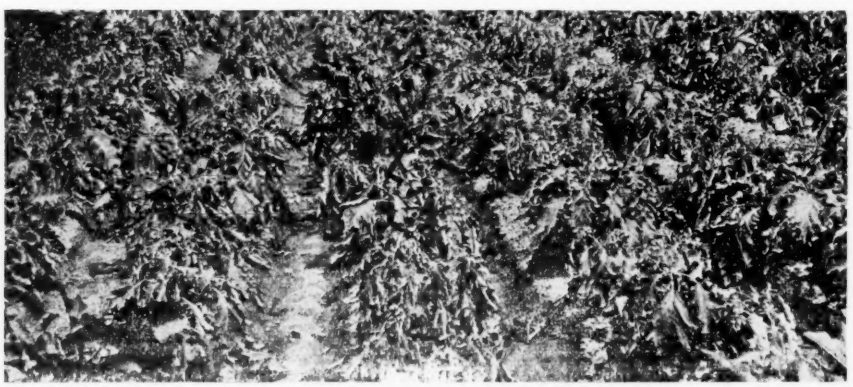

FIG. 1. EGGPLANT WILTED BY COLLETOTRICHUM SOLANICOLUM O'GARA. ONLY ONE PLANT (JUST ABOVE THE CROSS) IN THE FIELD WAS FREE FROM THE DISEASE

Mycologia.[2] At the time of publishing my earlier notes there was some doubt as to the parasitism of this new species. Cultures were exchanged

[1] O'Gara, P. J. A disease of the underground stems of Irish potato caused by a new species of Colletotrichum. Phytopath. **4**: 410–411. 1914.

[2] O'Gara, P. J. New species of Colletrotrichum and Phoma. Mycologia **7**: 38–41. 1915.

with Dr. J. J. Taubenhaus, then at the Delaware Agriculture College, who concluded that the organism is a species of Colletotrichum and that it has parasitic tendencies (oral communication).

During the summer of 1916 the writer had occasion to observe a field of eggplants in which fully ninety per cent of the plants showed wilt. At first it was supposed that the wilt was due to a Fusarium, noting only the general appearance of the field. A careful examination of the plants, however, indicated that Fusarium was not present but that the roots and stems were badly infected with the above-named organism. Interior portions of infected roots and stems were taken under sterile conditions and placed in culture tubes, where the organism fruited characteristically. No other organism appeared in the cultures where the proper precautions were taken. Even in mixed cultures, Fusarium did not appear.

When the organism was studied as a root and stem parasite of the potato, it was not suspected of being a serious wilt fungus, but in the case of the eggplant it has been found to produce a wilt disease similar to that produced by Fusarium. During the early growth of the plants no trouble was noted, but about the time some of the earlier fruits began to mature, the infected plants wilted. This condition shortly prevailed throughout the entire field, producing an almost total loss.

The field in which these eggplants were grown had supported a stand of potatoes the previous year and it was in this field of potatoes that the writer first found the above-named organism. From specimens collected in this field the original description was made.

This note is published in order to give notice of the economic character of this species of Colletotrichum.

P. J. O'GARA

Personals. Mr. K. E. Quantz, formerly assistant plant pathologist at the Virginia Experiment Station, has become plant pathologist to the Brazilian Government, with headquarters at Rio de Janeiro.

Mr. H. E. Thomas has resigned his position as instructor in plant pathology at the Virginia Polytechnic Institute to accept an appointment as assistant pathologist at the Federal Experiment Station at Mayaguez, Porto Rico.

Mr. Fred R. Jones, formerly a graduate student at the University of Wisconsin, has been appointed to the position of pathologist in charge of forage crop disease investigations, Bureau of Plant Industry, beginning April 14, 1917.

Mr. Gustav A. Meckstroth, a student at Pennsylvania State College, has accepted an appointment as scientific assistant in plant pathology, Office of Cotton, Truck and Forage Crop Disease Investigations, Department of Agriculture, beginning May 1, 1917.

LITERATURE ON PLANT DISEASES[1]

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY, AND FLORENCE P. SMITH, ASSISTANT

February to March, 1917

Arthur, Joseph Charles. Uredinales of Porto Rico based on collections by H. H. Whetzel and E. W. Olive. Mycologia **9**: 55–104. F. 1917.

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"A disease of the vine, vying in seriousness with oidium and phylloxera." Cause unknown.

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Blake, Maurice A., Cook, Melville Thurston, and **Schwarze, Carl Alois.** Studies on peach yellows and little peach. (Abstract.) Phytopathology **7**: 76–77. F. 1917.

Brock, W. S., and **Ruth, W. A.** Control of apple scab by bleaching powder. (Abstract.) Phytopathology **7**: 76. F. 1917.

Brooks, Charles, and **Cooley, Jacquelin Smith.** Jonathan spot. (Abstract.) Phytopathology **7**: 76. F. 1917.

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Buller, Arthur Henry Reginald. Black rust of wheat. Bul. Misc. Inform. Kew, **1917**: 48. F. 1917.

Economic loss due to wheat rust in North America.

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Dry root rot, *Fusarium* sp.; blight, *Bacterium Phaseoli*.

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[1] Beginning with this number, it is intended that this list shall include all references to the literature of plant diseases, both American and foreign. All foreign articles published since January 1, 1917, which come to our attention, will be entered, so that the index may be ultimately considered complete from that date.

All authors are urged to coöperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles published outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

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[PHYTOPATHOLOGY, for April, 1917 (**7:** 83–154, Pl. II) was issued April 5, 1917.]